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ance Notes on Codes and Abbreviations" appearing at the begin-
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(54) Title: METHODS FOR TREATING DEMYELINATING DISEASES

(57) Abstract: The invention provides a method of reducing the severity and reversing the neurological effects of a demyelinating
disease in a subject by administering to a subject having a demyelinating disease an effective amount of a neutralizing agent specific
for interferon inducible protein of 10 kDa (IP-10). The methods of the invention promote remyelination of demyelinated nerve fibers
and impede further demyyelination of nerve fibers in a subject affected with a demyelinating disease by administering to a subject
having a demyelinating disease.

METHODS FOR TREATING DEMYELINATING DISEASES

BACKGROUND OF THE INVENTION

This invention relates to immunology and, more specifically to treatment of demyelinating diseases through neutralization of the chemokine IP-10.

10   Demyelinating diseases are a group of disorders characterized by damage to the myelin sheath that coats and insulates nerve fibers. Individuals afflicted with a demyelinating disease suffer neurologial deficits due to impaired transmission of nerve impulses caused by myelin
15   being stripped from nerve fibers. Multiple Sclerosis, the most prominent of this group of diseases, is a neuroinflammatory, demyelinating disease of the central nervous system (CNS). MS is the leading cause of nontraumatic neurological disability among young adults
20   in North America and affects approximately one million young adults worldwide.

          MS usually affects multiple areas of white matter in the central nervous system (CNS), most frequently, the periventricular white matter, brainstem,
25   spinal cord and the optic nerves. The disease initially destroys myelin sheaths and eventually kills oligodendrocytes creating the characteristic plaque of MS. The early development of the plaque generally correlates with perivascular inflammation followed by the
30   migration of lymphocytes, plasma cells and macrophages

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into the lesion. Macrophage infiltration has been shown
to be directly related to the severity of nerve fiber
demyelination.

5   The incidence of MS and its pattern of
distribution have been unchanged for decades. The
disease usually presents in the from of recurrent attacks
of focal or multifocal neurologic dysfunction. Attacks
occur, remit, and recur, seemingly randomly over many
10   years. Remission is often incomplete and as one attack
follows another, a stepwise downward progression ensues
with increasing permanent deficit. Current MS management
includes interferons, corticosteroids and cytotoxic
immunosuppressive agents, often with unsatisfactory
15   outcomes, significant side effects and a lack of impact
on the chronic progression of MS. Current treatments can
hasten the recovery from acute exacerbations of MS by
reducing the inflammatory response, but generally do not
prevent future attacks, development of additional
20   disabilities or chronic progression of MS. Furthermore,
no treatments currently exist that reverse the
neurological impairments associated with demyelinating
diseases by causing remyelination of damaged nerve
fibers. As a result, supportive measures, symptomatic
25   treatment and comprehensive rehabilitation remain at the
core of management.

          Thus, there exists a need for treatments that
prevent the chronic progression and reverse the
neurological impairments of MS and other demyelinating
30   diseases. The present invention satisfies this need and
provides related advantages as well.

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SUMMARY OF THE INVENTION

The invention provides a method of promoting remyelination in a subject affected with a demyelinating disease by administering to a subject having a
5   demyelinating disease an effective amount of a neutralizing agent specific for interferon inducible protein of 10 kDa (IP-10). The invention also provides a method of reducing the severity of a demyelinating disease in a subject including remyelinating demyelinated
10   nerve fibers and impeding further demyelination by administering to a subject having a demyelinating disease an effective amount of a neutralizing agent specific for interferon inducible protein of 10 kDa (IP-10). Administration of an effective amount of a neutralizing
15   agent specific for interferon inducible protein of 10 kDa (IP-10) reduces the severity of a demyelinating disease by effecting the repair of demyelinating lesions in the nervous system and by impeding the ongoing demyelination associated with a demyelinating disease.

20   ## BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows increased mortality in anti-IP-10 treated mice. The mice were infected intracranially with MHV and treated intraperitoneally with either anti-IP-10 or normal rabbit serum (NRS).

25   Figure 2 shows delayed viral clearance from the CNS in anti-IP-10 treated mice. The number of mice examined is designated [a]n. The data presented represents three independent studies.

Figure 3 shows the decreased level of T
30   lymphocytes within the CNS of anti-IP-10 treated mice.

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Anti-IP-10 treatment of MHV infected mice resulted in a
82.3 percent decrease in infiltrating CD4+ T cells and a
70.4 percent decrease in infiltrating CD8+ T cells when
compared to NRS treatment of MHV infected mice. The data
5   are presented as a mean +/- standard error of the mean
(SEM) and represents the results of two independent
studies.

Figure 4 shows (A) analysis of IFN-γ mRNA
transcripts in anti-IP-10 treated mice at day 7 post
10   infection (*P≤0.05; NRS, n=3; anti-IP-10, n=3) and (B)
decreased expression of IFN-γ protein levels in anti-IP-
10 treated mice at day 7 post infection (*P≤0.01; NRS,
n=5; anti-IP-10, n=5).

Figure 5 shows the clinical scores of MHV-
15   infected mice treated with either anti-IP-10, anti-Mig or
NRS starting at day 12 post infection and ending at day
19 days infection. Clinical disease is assessed
according to a numerical scoring system based upon the
following parameters: 0 - no abnormality; 2 - limp tail
20   and partial hindlimb weakness, 3 - complete hindlimb
paralysis, 4 - death.

Figure 6 shows post treatment infiltration,
measured at day 28 post MHV infection, of CD4+ and CD8+ T
lymphocytes into the CNS of mice treated with anti-IP-10,
25   anti-Mig or NRS starting at day 12 post infection and
ending at day 19 post infection.

Figure 7 shows post treatment infiltration,
measured at day 21 post MHV infection, of CD4+ T
lymphocytes, CD8+ T lymphocytes and macrophages into the
30   CNS of mice treated with anti-IP-10, anti-Mig or NRS at

day 12 post infection and ending at day 19 post
infection.

Figure 8 shows demyelination at 21 days post
MHV infection in mice treated with either anti-IP-10,
5 anti-Mig or NRS starting at day 12 post infection and
ending at day 19 post infection (C.S., Clinical Score,
DM, Demyelination).

Figure 9 shows delayed viral clearance from the
CNS in anti-Mig treated mice. The number of mice
10 examined is designated n.

Figure 10 shows Mig expression at day 7 post
infection and day 12 post infection in the CNS of MHV
infected mice. The data are presented as a mean ± SEM
(PID 7 n=3; PID 12 n=2; PID 35 NRS n=3)

15   Figure 11 shows the increased mortality in
anti-Mig treated mice. For the anti-Mig group n=27 and
for the NRS group n=27.

Figure 12 shows decreased levels of
infiltrating T lymphocytes within the CNS of anti-Mig
20 treated mice. The data are presented as a mean ± SEM and
represent the results of two independent studies. For
the NRS group n=5 and for the anti-Mig group n=5.

Figure 13 shows cytokine expression in brains
of MHV infected mice treated with either anti-Mig or NRS.
25 Data are presented as mean ± SEM (*p ≤ 0.02). For the
NRS group n=3 and for the anti-Mig group n=3.

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Figure 14 shows decreased expression of IFN-γ protein in anti-Mig treated mice. Data are presented as mean ± SEM (*p ≤ 0.05). For the NRS group n=5 and for the anti-Mig group n=5.

5   Figure 15 shows increased expression of IL-10 protein in anti-Mig treated mice. Data are presented as mean ± SEM (*p ≤ 0.02). For the NRS group n=5 and for the anti-Mig group n=5.

Figure 16 shows (A) remyelination of viral-
10 induced nerve fiber demyelination by anti-IP 10 treatment, and (B) viral-induced nerve fiber demyelination without anti-IP 10 treatment.

DETAILED DESCRIPTION OF THE INVENTION

This invention is directed to a method of
15 treating demyelinating diseases including Multiple Sclerosis (MS) by neutralizing interferon inducible protein of 10 kDA (IP-10) with a specific neutralizing agent. Neutralization of IP-10 represents a significant treatment option for demyelinating diseases because it
20 causes the remyelination of demyelinated nerve fibers, thereby reversing the neurological impairments associated with demyelination, and further impedes the nerve fiber demyelination that is responsible for the neurological impairments associated with demyelinating diseases.
25 Significantly, neutralization of IP-10 targets a cause rather than merely the symptoms of neurological impairments associated with demyelination.

In another embodiment, the invention is directed to a method of treating demyelinating diseases
30 including Multiple Sclerosis (MS) by neutralizing

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monokine induced by interferon-γ (Mig) with a specific
neutralizing agent.

　　　　As used herein, the term "demyelinating
disease" is intended to refer to a disease or condition
5   that is characterized by the loss of the myelin sheath
surrounding the nerve fibers (axons) of nerve cells.
Inflammation can accompany the loss of myelin in
demyelinating diseases. The clinical course of a
demyelinating disease can be, for example, acute, chronic
10   or remitting and relapsing. The loss of myelin in a
demyelinating disease can occur in the central nervous
system (CNS), the peripheral nervous system (PNS) or
both. For example, Multiple Sclerosis (MS) is a
demyelinating disease of the central nervous system (CNS)
15   in which demyelination occurs in the white matter of the
brain and spinal cord. Acute demyelinating
polyneuropathy, better known as Guillain-Barre syndrome
(GBS) and its variant chronic inflammatory demyelinating
polyneuropathy (CIDP) are examples of demyelinating
20   diseases of the peripheral nervous system.

　　　　As used herein, the term "neuroinflammatory
disease" refers to a disease that is characterized by T-
cell infiltration into the central nervous system.
Neuroinflammatory conditions include both demyelinating
25   diseases and non-demyelinating inflammatory diseases, for
example, viral encephalitis, bacterial encephalitis and
bacterial meningitis. Viral encephalitis can be caused
by a variety of infectious agents including, for example,
Japanese encephalitis virus, HIV-1 and cytomegalovirus.

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As used herein, the term "remyelination" refers to a gross increase in the myelination of demyelinated nerve fibers. A gross increase in myelination can be detected through a variety of methods known in the art

5   As used herein, the term "effective amount" when used in reference to interferon inducible protein of 10 kDa (IP-10), is intended to mean an amount of a neutralizing agent specific for IP-10 sufficient to reduce the severity of a demyelinating disease or

10   sufficient to promote remyelination in a subject affected with a demyelinating disease. Similarly, the term "effective amount" when used in reference to monokine induced by interferon-γ (Mig) is intended to mean an amount of a neutralizing agent specific for Mig

15   sufficient to reduce the severity of a demyelinating disease.

As used herein, "reduction in severity" is intended to refer to an arrest, decrease or reversal in signs and symptoms, physiological indicators, biochemical

20   markers or metabolic indicators associated with a particular disease. Symptoms of a demyelinating disease include, for example, neurological impairments and neuroinflammation. Physiological indicators of a demyelinating disease include the demyelination of nerve

25   fibers. Biochemical markers of a demyelinating disease are, for example, myelin, gamma globulin or the specific molecules that give rise to oligoclonal banding. Demyelination as well as remyelination of nerve fibers can be detected by a variety of clinical methods well

30   known in the art. For example, evoked potentials (EP) can be used to measure how quickly nerve impulses travel along the nerve fibres in various parts of the nervous system. In addition, computer-assisted tomography (CT)

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can be used to scan the central nervous system to detect
areas of demyelination as well as remyelination of nerve
fibers. Magnetic resonance imaging (MRI) also can be
used to scan the central nervous system, but without the

5   use of x-rays. More sensitive than the CT scan, MRI can
detect areas of demyelination as well as remyelination
that may not be seen by the CT scanner. Moreover, lumbar
puncture or spinal tap procedures can be used to draw out
cerebrospinal fluid. The fluid is examined for increased

10 levels of gamma globulin and oligoclonal banding. These
and other methods well known in the art can be used to
determine the severity of a demyelinating disease by
measuring myelination changes such as demyelination as
well as remyelination of nerve fibers.

15   As used herein, the term "neutralizing agent
specific for interferon inducible protein of 10 kDa (IP-
10)" is intended to refer to an agent effecting a
decrease in the extent, amount or rate of IP-10
expression or effecting a decrease in the activity of IP-

20 10. Similarly, as used herein, the term "neutralizing
agent specific for monokine induced by interferon-γ
(Mig)" is intended to refer to an agent effecting a
decrease in the extent, amount or rate of Mig expression
or effecting a decrease in the activity of Mig.

25 Neutralizing agents useful for practicing the claimed
invention include, for example, binding molecules such as
antibodies against IP-10 or Mig as well as molecules that
modulate or regulate IP-10 or Mig through non-binding
interactions. A neutralizing agent can be, for example,

30 any molecule that binds IP-10 or Mig with sufficient
affinity to decrease IP-10 or Mig activity, respectively.
Additionally, a neutralizing agent can be any molecule
binds to a regulatory molecule or gene region so as to
inhibit or promote the function of the regulatory protein

or gene region and effect a decrease in the extent or amount or rate of IP-10 or Mig expression or activity. Thus, a neutralizing agent can be any molecule that directly or indirectly modulates or regulates the extent,

5   amount or rate of IP-10 or Mig expression or activity. For example, a fragment or peptidomimetic of the CXCR3 receptor that binds IP-10 and Mig with sufficient affinity to decrease IP-10 or Mig activity, respectively, is useful for practicing the claimed methods. In

10 addition, examples of neutralizing agents which effect a decrease in IP-10 or Mig expression can include antisense nucleic acids and transcriptional inhibitors. As used herein, the term "candidate" refers to a neutralizing agent prior to confirmation of its neutralizing activity.

15   The invention provides a method of promoting remyelination in a subject affected with a demyelinating disease. The method consists of administering to a subject having a demyelinating disease an effective amount of a neutralizing agent specific for interferon

20 inducible protein of 10 kDa (IP-10). The invention further provides a method of reducing the severity of a demyelinating disease. The method comprises remyelinating demyelinated nerve fibers and impeding further demyelination of affected nerve fibers by

25 administering to a subject having a demyelinating disease an effective amount of a neutralizing agent specific for interferon inducible protein of 10 kDa (IP-10).

        The invention also provides a method of reducing the severity of a neuroinflammatory disease in a

30 subject, comprising administering to a subject having a neuroinflammatory disease an effective amount of a neutralizing agent specific for interferon inducible protein of 10 kDa (IP-10).

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The invention further provides a method of
inhibiting T-cell migration into the central nervous
system (CNS) associated with a neuroinflammatory disease,
comprising administering to a subject an effective amount
5   of a neutralizing agent specific for interferon inducible
protein of 10 kDa (IP-10).

In addition, the invention provides a method of
reducing the severity of a demyelinating disease in a
subject, comprising administering to a subject having a
10 demyelinating disease an effective amount of a
neutralizing agent specific for monokine induced by γ-
interferon (Mig).

A successful host response to inflammation
generally requires the accumulation of specialized host
15 cells at the site of tissue damage. This cellular
accumulation is a critical step in the normal
inflammatory process and is mediated by a family of
secreted chemotactic cytokines that have in common
important structural features and a role in pathogenic
20 inflammation. IP-10 and Mig belong to this protein
family of over forty structurally and functionally
related proteins known as chemokines.

Chemokines are homologous 8 to 14 kDa heparin
binding proteins that possess a conserved structural
25 motif containing two cysteine pairs and are divided into
subfamilies based on the relative position of the
cysteine residues in the mature protein. There are at
least four subfamilies of chemokines, but only two, α-
chemokines and β-chemokines, have been well
30 characterized. In the α-chemokines, the first two
cysteine residues are separated by a single amino acid
(CXC), whereas in the β-chemokines the first two cysteine

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residues are adjacent to each other (CC). The C-X-C
chemokines include, for example, interleukin-8 (IL-8),
human platelet derived factors, IP-10 and Mig. Members
of the C-C chemokine subfamily include, for example,

5   macrophage chemoattractant protein (MCP), macrophage
inflammatory protein-1α (MIP-1α), macrophage inflammatory
protein-1β (MIP-1β) and regulated on activation, normal
T-cell expressed and secreted (RANTES).

     Chemokines selectively attract leukocyte
10   subsets; some chemokines act specifically toward
eosinophils, others toward monocytes, dendritic cells, or
T cells (see Luster, A., New Engl. J. Med. 338: 436-445
(1998), which is incorporated herein by reference). In
general, CC chemokines chemoattract monocytes,
15   eosinophils, basophils, and T cells; and signal through
the chemokine receptors CCR1 to CCR9. The CXC chemokine
family can be further divided into two classes based on
the presence or absence of an ELR sequence (Glu-Leu-Arg)
near the N terminal preceding the CXC sequence. The
20   ELR-containing CXC chemokines including IL-8 chemoattract
neutrophils, while the non-ELR CXC chemokines including
IP-10 and Mig chemoattract T lymphocytes.

     Chemokines induce cell migration and
activation, in part, by binding to specific G-protein
25   coupled cell-surface receptors on target cells. More
than 10 distinct chemokine receptors, each expressed on
different subsets of leukocytes, have been identified.
Chemokine receptors are constitutively expressed on some
cells, whereas they are inducible on others. CXCR3, the
30   receptor recognized by IP-10 and Mig, is expressed on
activated T lymphocytes of the T helper type 1 (Th1)
phenotype and Natural Killer (NK) Cells. Significantly,
a central mechanism in the pathology of neuroinflammatory

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diseases, including both demyelinating and non-demyelinating neuroinflammatory diseases, is the organ-specific migration of activated T cells into the central nervous system.

5   Demyelinating diseases are an important group of neurological disorders because of the frequency with which they occur and the disability that they cause. Demyelinating diseases have in common a focal or patchy destruction of myelin sheaths that can be accompanied by

10 an inflammatory response. Myelin loss also occurs in other conditions, for example, in genetically determined defects in myelin metabolism, and as a consequence of toxin exposure and infections of oligodendrocytes or Schwann cells.

15   Demyelinating diseases can be divided into processes affecting myelin of the central nervous system and those affecting myelin of the peripheral nervous system. Demyelinating diseases of the central nervous system include, for example, MS, acute disseminated

20 encephalomyelitis (ADE) including postinfectious and postvaccinal encephalomyelitis, acute necrotizing hemorrhagic encephalomyelitis and progressive (necrotizing) myelopathy. Demyelinating diseases of the peripheral nervous sytem include, for example, acute

25 inflammatory demyelinating polyradiculoneuropathy (Guillain-Barré syndrome), chronic inflammatory demyelinating polyradiculoneuropathy (CIDP), demyelinating neuropathy associated with IgM monoclonal gammopathy and neuropathy associated with sclerosing

30 myeloma.

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The methods of the invention can be practiced with demyelinating diseases regardless of their etiology. MS is a central nervous system demyelinating disease with a likely autoimmune etiology as reviewed in Martin et

5   al., _Annu. Rev. Immunol_. 10:153-187 (1992), which is incorporated herein by reference. In contrast, ADE is a central nervous system demyelinating disease that develops following an infectious illness, often as a sequela of measles. Despite their distinct etiologies

10 both, MS and ADE as well as other demyelinating diseases known to those skilled in the art, including those described herein are appropriate therapeutic targets for the methods of the invention based on the shared feature of inflammatory demyelination characterized by migration

15 of lymphocytes and macrophages to the site of tissue damage. As set forth above, a central mechanism in the pathology of neuroinflammatory demyelinating diseases is the organ-specific migration of activated T cells into the central nervous system. Based on this shared disease

20 mechanism, any neuroinflammatory disease that is characterized by T-cell infiltration of the central nervous system is a therapeutic target for practicing the methods of the invention. Thus, methods of the invention also can be used to reduce the severity of a non-

25 demyelinating neuroinflammatory disease including, for example, viral encephalitis, bacterial encephalitis and bacterial meningitis. Viral encephalitis can be caused by a variety of infectious agents including, for example, Japanese encephalitis virus, HIV-1 and cytomegalovirus.

30 Bacterial meningitis can be caused, for example, by listeria. It is contemplated that the methods of the invention directed to reducing the severity of an inflammatory disease are applicable regardless of the specific type of infectious agent.

As described herein, IP-10 and Mig are
components in mounting a host defense against
inflammation of the nervous system. Specifically, as
shown herein, the IP-10 and Mig coordinate the
5   trafficking of Th1 T lymphocytes into the CNS in response
to a pathological aberration such as an infectious agent
or autoagressive immune cells. T lymphocytes can be
subdivided into two major categories known as CD4$^+$ cells
and CD8$^+$ cells. CD4 and CD8 are surface proteins that
10   facilitate interactions between T cell receptors for
antigen and antigen itself which is presented to T cells
by antigen-presenting cells. Antigens recognized by T
cells are contained within clefts of major
histocompatibility complex (MHC) proteins expressed on
15   the surface of antigen-presenting cells. CD4 cells
recognize peptide fragments presented by class II
histocompatibility alleles, CD8 cells recognize fragments
presented by class I histocompatibility alleles. The DR2
allele, over-represented in multiple sclerosis, is a MHC
20   class II allele, pointing to a role for CD4$^+$ cells in
lesion formation in multiple sclerosis.

CD4$^+$ cells can be further subdivided into Th1
and Th2 subtypes. Th1 cells are responsible for delayed
type hypersensitivity responses and secrete numerous
25   cytokines including interleukin-2 (IL-2), a stimulator of
T cell proliferation, and interferon, an activator of
macrophages, and lymphotoxin, a protein which has the
capacity to damage oligodendrocytes, the myelin-forming
cells of the CNS. Interferon administration provokes
30   multiple sclerosis attacks, and elevated numbers of
IF-secreting cells can be detected in the blood during
attacks and in MS plaques. Interferon, in combination
with IL-2 activates macrophages which directly strip
myelin from nerve fibers as well as sectrete tumor

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necrosis factor-α (TNF-α), a cytokine that damages the
myelin-producing oligodendrocytes.

As described herein, neutralization of IP-10,
which is expressed during the chronic stages of
5   neurological disease almost exclusively in the area of
demyelination, causes remyelination of demyelinated nerve
fibers and reduces the further progression of
demyelination (Figure 8). As such, neutralizing IP-10
activity can lead to a reduction in the severity of the
10 demyelinating disease as well as to the level of
neurological impairments associated with demyelination.
IP-10 neutralizing agents including antibodies, antisense
nucleic acids and compounds identified by the methods
described below are useful for treating or reducing the
15 severity of a demyelinating disease and for promoting
remyelination in a subject affected with a demyelinating
disease.

An IP-10 or Mig specific neutralizing agent
modulates IP-10 or Mig, respectively, sufficiently to
20 reduce activity of IP-10 or Mig related to cell
recruitment, demyelination and the prevention of
remyelination. An IP-10 or Mig specific neutralizing
agent can modulate the activity of IP-10 and Mig both
through binding and non-binding interactions. An IP-10
25 or Mig specific neutralizing agent can be a
macromolecule, such as polypeptide, nucleic acid,
carbohydrate or lipid. An IP-10 and Mig specific
neutralizing agent can also be a derivative, analogue or
mimetic compound as well as a small organic compound as
30 long as IP-10 or Mig activity is reduced in the presence
of the neutralizing agent. The types of molecules that
can be neutralizing agents useful for practicing the
methods of the invention as well as the methods of

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administration described herein with reference to either
IP-10 or Mig are equally applicable to both chemokines.
The size of a neutralizing agent is not important so long
as the molecule exhibits or can be made to exhibit

5   selective neutralizing activity towards IP-10 or Mig.
For example, a neutralizing agent can be as little as
between about one and six, and as large as tens or
hundreds of monomer building blocks which constitute a
macromolecule or chemical binding molecule. Similarly,

10   an organic compound can be a simple or complex structure
so long as it binds IP-10 or MIG with sufficient affinity
to reduce activity.

     Neutralizing agents specific for IP-10 or Mig
can include, for example, antibodies and other receptor

15   or ligand binding polypeptides of the immune system.
Such other molecules of the immune system include for
example, T cell receptors (TCR) including CD4 cell
receptors. Additionally, cell surface receptors such as
integrins, growth factor receptors and chemokine

20   receptors, as well as any other receptors or fragments
thereof that bind IP-10 or Mig, or can be made to bind
IP-10 or Mig, with sufficient affinity to reduce activity
are also neutralizing agents useful for practicing the
methods of the invention. Additionally, receptors,

25   growth factors, cytokines or chemokines, for example,
which inhibit the expression of IP-10, Mig or their
receptors are also neutralizing agents useful for
practicing the methods of the invention. Furthermore,
DNA binding polypeptides such as transcription factors

30   and DNA replication factors are likewise included within
the definition of the term binding molecule so long as
they have selective binding activity for IP-10, Mig, or
regulatory molecules that control the expression or
activity of IP-10 or Mig or gene regions that control the

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expression of IP-10 or Mig. Finally, polypeptides,
nucleic acids and chemical compounds such as those
selected from random and combinational libraries are also
included within the definition of the term so long as
5   such a molecule binds IP-10 or Mig with sufficient
affinity to decrease activity.

        Various approaches can be used for identifying
neutralizing agents selective for either IP-10 or Mig.
For example, one approach is to use the information
10 available regarding the structure and function of IP-10,
Mig or a modulator of IP-10 or Mig, to binding molecule
populations, from molecules known to function as
chemokine binding molecules or known to exhibit or be
capable of exhibiting binding affinity specific for IP-
15 10, Mig or a modulator of IP-10 or Mig, such as fragments
or mimetics of the CXCR3 receptor found on CD4+ T cells
and NK cells. A neutralizing agent specific for IP-10 or
Mig can be an antibody and other receptor of the immune
repertoire. The normal function of such immune receptors
20 is to bind essentially an infinite number of different
antigens and ligands. Therefore, generating a diverse
population of binding molecules from an immune
repertoire, for example, can be useful for identifying a
neutralizing agent specific for IP-10 or Mig. A binding
25 molecule useful in the methods of the invention also can
be selective for a molecule that regulates or modulates
IP-10 or Mig expression and activity.

        A neutralizing agent specific for IP-10 or Mig
can further be identified from a large population of
30 unknown molecules by methods well known in the art. Such
a population can be a random library of peptides or small
molecule compounds. The population can be generated to
contain a sufficient diversity of sequence or structure

so as to contain a molecule which will bind to the IP-10
or Mig protein or their respective nucleic acids. Those
skilled in the art will know what size and diversity is
necessary or sufficient for the intended purpose. A

5 population of sufficient size and complexity can be
generated so as to have a high probability of containing
an IP-10 or Mig neutralizing agent that binds IP-10 or
Mig with sufficient affinity to decrease activity.
Numerous other types of library molecule populations

10 exist and are described further below.

Any molecule that modulates or regulates IP-10
activity or expression, an IP-10 receptor activity or
expression, a gene region that controls IP-10 expression,
or a regulatory molecule that modulates IP-10 activity or

15 expression as well as any regulatory molecule that
modulates or regulates IP-10 receptor expression is an
IP-10 specific neutralizing agent useful for practicing
the invention. For example, any molecule that binds to
IP-10, to an IP-10 receptor, to a gene region that

20 controls IP-10 expression, or to a regulatory molecule
that modulates IP-10 activity or expression as well as to
any regulatory molecule that modulates IP-10 receptor
expression is an IP-10 specific neutralizing agent useful
for practicing the invention. Thus, an IP-10 specific

25 neutralizing agent can act directly through a binding or
non-binding interaction with IP-10 as well as indirectly
through a binding or non-binding interaction with any
other molecule that regulates or modulates IP-10
expression or activity.

30 Similarly, any molecule that modulates or
regulates Mig activity or expression, an Mig receptor
activity or expression, a gene region that controls Mig
expression, or a regulatory molecule that modulates Mig

20

activity or expression as well as any regulatory molecule
that modulates or regulates Mig receptor expression is an
Mig specific neutralizing agent useful for practicing the
invention. For example, any molecule that binds to Mig,

5   to a Mig receptor, to a gene region that controls Mig
expression, or to a regulatory molecule that modulates
Mig activity or Mig expression as well as to any
regulatory molecule that modulates Mig receptor
expression is a Mig specific neutralizing agent useful

10 for practicing the invention. Thus, an Mig specific
neutralizing agent can act directly through a binding or
non-binding interaction with Mig as well as indirectly
through a binding or non-binding interaction with any
other molecule that regulates or modulates Mig expression

15 or activity. An IP-10 or Mig specific neutralizing agent
can be a regulatory molecule affects IP-10 or Mig
expression by reducing or inhibiting the action of a
transcription factor that controls or upregulates
transcription of IP-10 or Mig, respectively. In

20 addition, a regulatory molecule that binds with
sufficient affinity to a molecule involved in the
activation of IP-10 or Mig to reduce IP-10 or Mig
activation is a neutralizing agent useful for practicing
the methods of the invention.

25   A neutralizing agent can modulate IP-10 or Mig
expression or activity sufficiently to decrease their
activity is useful for practicing the claimed methods of
reducing the severity of a demyelinating disease and
promoting remyelination in a subject affected with a

30 demyelinating disease. In addition, an IP-10 or Mig
specific neutralizing agent can decrease IP-10 or Mig
activity or expression by modulating an IP-10 or Mig
receptor, a regulatory molecule that, in turn, modulates
the activity or expression of IP-10 or Mig, or a gene

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region that controls IP-10 or Mig expression. For
example, a neutralizing agent useful for practicing the
claimed invention can be an antibody against a regulator
molecule that modulates IP-10 or Mig expression or

5   activity. Furthermore, as described herein, an anti-IP-
10 or anti-Mig-antibody is a useful neutralizing agent
for practicing the methods of the invention. In
addition, since IP-10 and Mig are secreted proteins known
to bind to a specific T-cell receptor, a neutralizing

10   agent useful for practicing the invention can be any
binding polypeptide, receptor or fragment thereof of the
immunoglobulin superfamily of receptors. Alternatively,
it may be desired to use populations of random peptide
populations to identify further neutralizing agents

15   specific for IP-10 or Mig. Those skilled in the art will
know or can determine what type of approach and what type
of neutralizing agent is appropriate for practicing the
methods of the invention for reducing the severity of a
demyelinating disease and promoting remyelination in a

20   subject affected with a demyelinating disease.

A moderate sized population for identification
of an IP-10 or Mig-specific neutralizing agent can
consist of hundreds and thousands of different molecules,
for example, binding molecules, within the population

25   whereas a large population will consist of tens of
thousands and millions of different molecule species.
More specifically, large and diverse populations of
molecules for the identification of a neutralizing agent
will contain any of about 10^4, 10^5, 10^6, 10^7, 10^8, 10^9,

30   10^{10}, or more, different molecule species. One skilled in
the art will know the approximate diversity of the
population of molecules sufficient to identify a
neutralizing agent that modulates or regulates IP-10 or
Mig activity or expression.

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Recombinant libraries of molecules, for
example, binding molecules, can be used to identify a
neutralizing agent specific for IP-10 or Mig since large
and diverse populations can be rapidly generated and
5   screened with IP-10 and Mig. Recombinant libraries of
expressed polypeptides useful for identifying a
neutralizing agent specific for IP-10 or Mig can be
engineered in a large number of different ways known in
the art. Recombinant library methods similarly allow for
10 the production of a large number of binding molecule
populations from naturally occurring repertoires.
Whether recombinant or otherwise, essentially any source
of binding molecule population can be used so long as the
source provides a sufficient size and diversity of
15 different binding molecules to identify a neutralizing
agent specific for IP-10 or Mig. If desired, a
population of binding molecules useful for identifying a
neutralizing agent specific for IP-10 or Mig can be a
selectively immobilized to a solid support as described
20 by Watkins et al., Anal. Biochem. 256 (92): 169-177
(1998), which is incorporated herein by reference.

A phage expression library in which lysogenic
phage cause the release of bacterially expressed binding
molecule polypeptides is a specific example of a
25 recombinant library that can be used to identify a
neutralizing agent specific for IP-10 or Mig. In another
type of phage expression library, large numbers of
potential binding molecules can be expressed as fusion
polypeptides on the periplasmic surface of bacterial
30 cells. Libraries in yeast and higher eukaryotic cells
exist as well and are similarly applicable in the methods
of the invention. Those skilled in the art will know or

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can determine what type of library is useful for
identifying a neutralizing agent specific for IP-10 or
Mig.

5   In addition to the methods described above,
which utilize purified polypeptide to screen libraries of
compounds for those which specifically bind IP-10 or Mig,
a neutralizing agent specific for IP-10 or Mig can be
identified by using purified polypeptide to produce
antibodies. For example, antibodies which are specific
10   for IP-10 can be used as neutralizing agents of the
invention and can be generated using methods that are
well known in the art. Neutralizing agents useful for
practicing the methods of the invention include both
polyclonal and monoclonal antibodies against IP-10, Mig,
15   or any molecule that modulates IP-10 or Mig expression or
activity, as well as antigen binding fragments of such
antibodies including Fab, F(ab')2, Fd and Fv fragments
and the like. In addition, neutralizing agents useful
for practicing the methods of the invention encompass
20   non-naturally occurring antibodies, including, for
example, single chain antibodies, chimeric antibodies,
bifunctional antibodies, complementarity determining
region-grafted (CDR-grafted) antibodies and humanized
antibodies, as well as antigen-binding fragments thereof.

25   Methods of preparing and isolating antibodies,
including polyclonal and monoclonal antibodies, using
peptide immunogens, are well known to those skilled in
the art and are described, for example, in Harlow and
Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor
30   Laboratory Press (1988). Non-naturally occurring
antibodies can be constructed using solid phase peptide
synthesis, can be produced recombinantly or can be
obtained, for example, by screening combinatorial

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libraries consisting of variable heavy chains and
variable light chains as described by Huse et al.,
Science 246:1275-1281 (1989), which is incorporated
herein by reference. These and other methods of making,
5   for example, chimeric, humanized, CDR-grafted, single
chain, and bifunctional antibodies are well known to
those skilled in the art (Hoogenboom et al., U.S. Patent
No. 5,564,332, issued October 15, 1996; Winter and
Harris, Immunol. Today 14:243-246 (1993); Ward et al.,
10   Nature 341:544-546 (1989) ; Harlow and Lane, Antibodies:
A laboratory manual (Cold Spring Harbor Laboratory Press,
1988); Hilyard et al., Protein Engineering: A practical
approach (IRL Press 1992); Borrabeck, Antibody
Engineering, 2d ed. (Oxford University Press 1995); each
15   of which is incorporated herein by reference).

An IP-10 or Mig specific antibody neutralizing
agent can be raised using as an immunogen a substantially
purified IP-10 or Mig protein, which can be prepared from
natural sources or produced recombinantly, or a peptide
20   portion of an IP-10 or Mig protein including synthetic
peptides. A non-immunogenic peptide portion of a IP-10
or Mig protein can be made immunogenic by coupling the
hapten to a carrier molecule such bovine serum albumin
(BSA) or keyhole limpet hemocyanin (KLH), or by
25   expressing the peptide portion as a fusion protein.
Various other carrier molecules and methods for coupling
a hapten to a carrier molecule are well known in the art
(see Harlow and Lane, supra, 1988; see, also, Hermanson,
Bioconjugate Techniques, Academic Press, 1996, which is
30   incorporated herein by reference). As described above,
an antibody neutralizing agent specific for IP-10 or Mig
can also be raised against a regulatory molecule that
modulates IP-10 or Mig expression or activity rather than
against IP-10 or Mig directly.

A neutralizing agent specific for either IP-10
or Mig, such as an antibody can be labeled so as to be
detectable using methods well known in the art
(Hermanson, supra, 1996; Harlow and Lane, supra,1988;
5   chap. 9). For example, a neutralizing agent specific for
IP-10 or Mig can be linked to a radioisotope or
therapeutic agent by methods well known in the art. A
neutralizing agent that directly binds IP-10 or Mig
linked to a radioisotope or other moiety capable of
10 visualization can be useful to diagnose or stage the
progression of a clinical stage of a demyelinating
disease or neuroinflammatory disease that is
characterized by the organ or tissue-specific presence or
absence of IP-10 or Mig. Thus, the invention also
15 provides diagnostic methods applicable to conditions that
are associated with IP-10 or Mig activity or expression.

Methods for raising polyclonal antibodies, for
example, in a rabbit, goat, mouse or other mammal, are
well known in the art (Harlow and Lane, supra, 1988).
20 The production of anti-peptide antibodies commonly
involves the use of host animals such as rabbits, mice,
guinea pigs, or rats. If a large amount of serum is
needed, larger animals such as sheep, goats, horses,
pigs, or donkeys can be used. Animals are usually chosen
25 based on the amount of antiserum required and suitable
animals include rabbits, mice, rats, guinea pigs, and
hamsters. These animals yield a maximum of 25 mL,
100-200 µL, 1-2 mL, 1-2 mL, and 1-2 mL of serum per
single bleed, respectively (Harlow and Lane, supra,
30 1988). Rabbits are very useful for the production of
polyclonal antisera, since they can be safely and
repeatedly bled and produce high volumes of antiserum.
Two injections two to four weeks apart with 15-50µg of
antigen in a suitable adjuvant such as, for example,

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Freund's Complete Adjuvant can be followed by blood
collection and analysis of the antiserum.

5   In addition, monoclonal antibodies can be
obtained using methods that are well known and routine in
the art (Harlow and Lane, supra, 1988). A peptide
portion of a protein such as IP-10 or Mig for use as an
immunogen can be determined by methods well known in the
art. Spleen cells from an IP-10 or Mig immunized mouse
can be fused to an appropriate myeloma cell line to
10   produce hybridoma cells. Cloned hybridoma cell lines can
be screened using a labeled IP-10 or Mig protein to
identify clones that secrete anti-IP-10 or anti-Mig
antibodies, respectively. Hybridomas expressing anti-IP-
10 or anti-Mig monoclonal antibodies having a desirable
15   specificity and affinity can be isolated and utilized as
a continuous source of the antibody neutralizing agent.

      Neutralizing agents specific for IP-10 or Mig
can be used to reduce the severity of a demyelinating
disease in a mammal. Neutralizing agents specific for
20   IP-10 or Mig can promote remyelination in a subject
affected with a demyelinating disease in a mammal. Thus,
neutralizing agents specific for IP-10 or Mig can be used
to reduce the severity of a demyelinating disease in a
human subject. Furthermore, neutralizing agents specific
25   for IP-10 or Mig can be used to promote remyelination in
a subject affected with a demyelinating disease in a
human subject. Humanized antibodies can be constructed
by conferring essentially any antigen binding specificity
onto a human antibody framework. Methods of constructing
30   humanized antibodies are useful to prepare an antibody
neutralizing agent appropriate for practicing the methods
of the invention and avoiding host immune responses

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against the antibody neutralizing agent when used
therapeutically.

The antibody neutralizing agents described
above can be used to generate therapeutic human
5   neutralizing agents by methods well known in the art such
as complementary determining region (CDR)-grafting and
optimization of framework and CDR residues. For example,
humanization of an antibody neutralizing agent can be
accomplished by CDR-grafting as described in Fiorentini
10   at al., Immunotechnology 3(1): 45-59 (1997), which is
incorporated herein be reference. Briefly, CDR-grafting
involves recombinantly splicing CDRs from a nonhuman
antibody neutralizing agent into a human framework region
to confer binding activity onto the resultant grafted
15   antibody, or variable region binding fragment thereof.
Once the CDR-grafted antibody, or variable region binding
fragment is made, binding affinity comparable to the
nonhuman antibody neutralizing agent can be reacquired by
subsequent rounds of affinity maturation strategies known
20   in the art. Humanization of antibody neutralizing agents
in the form of rabbit polyclonal antibodies can be
accomplished by similar methods as described in Rader et
al., J. Biol. Chem. 275(18): 13668-13676 (2000), which is
incorporated herein be reference.

25   Humanization of a nonhuman IP-10 or Mig
antibody neutralizing agent can also be achieved by
simultaneous optimization of framework and CDR residues,
which permits the rapid identification of co-operatively
interacting framework and CDR residues, as described in
30   Wu et al., J. Mol. Biol. 294(1): 151-162 (1999), which is
incorporated herein by reference. Briefly, a
combinatorial library that examines a number of
potentially important framework positions is expressed

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concomitantly with focused CDR libraries consisting of variants containing random single amino acid mutations in the third CDR of the heavy and light chains. By this method, multiple Fab variants containing as few as one nonhuman framework residue and displaying up to approximately 500-fold higher affinity than the initial chimeric Fab can be identified. Screening of combinatorial framework-CDR libraries permits identification of monoclonal antibodies with structures optimized for function, including instances in which the antigen induces conformational changes in the monoclonal antibody. The enhanced humanized variants contain fewer nonhuman framework residues than antibodies humanized by sequential in vitro humanization and affinity maturation strategies known in the art.

It is further contemplated that a neutralizing agent of the invention can be a human antibody or a primatized antibody. In addition, human antibodies can be produced by methods known in the art that involve immunizing a transgenic nonhuman animal with the desired antigen. The transgenic nonhuman animal can be modified such that it fails to produce endogenous antibodies, but instead produces B-cells which secrete fully human immunoglobulins. The antibodies produced can be obtained from the animal directly or from immortalized B-cells derived from the transgenic nonhuman animal. Alternatively, the genes encoding the immunoglobulins with human variable regions can be recovered and expressed to obtain the antibodies directly or modified to obtain analogs of antibodies such as, for example, single chain F_v molecules. Thus, it is contemplated to produce a neutralizing agent of the invention that is a fully human immunoglobulin to a specific antigen or to produce an analog of the immunoglobulin by a process that

includes immunizing a nonhuman animal with antigen under
conditions that stimulate an immune response.

The nonhuman animal that produces a human
antibody neutralizing agent of the invention can be
5   modified to be substantially incapable of producing
endogenous heavy or light immunoglobulin chain, but
capable of producing immunoglobulins with both human
variable and constant regions. In the resulting immune
response, the animal produces B cells which secrete
10   immunoglobulins that are fully human and specific for the
antigen. The human immunoglobulin of desired specificity
can be directly recovered from the animal, for example,
from the serum, or primary B cells can be obtained from
the animal and immortalized. The immortalized B cells
15   can be used directly as the source of human antibodies
or, alternatively, the genes encoding the antibodies can
be prepared from the immortalized B cells or from primary
B cells of the blood or lymphoid tissue, for example,
spleen, tonsils, lymph nodes, bone marrow, of the
20   immunized animal and expressed in recombinant hosts, with
or without modifications, to produce the neutralizing
agent immunoglobulin or its analogs. In addition, the
genes encoding the repertoire of immunoglobulins produced
by the immunized animal can be used to generate a library
25   of immunoglobulins to permit screening for those variable
regions which provide the desired affinity. Clones from
the library which have the desired characteristics can
then be used as a source of nucleotide sequences encoding
the desired variable regions for further manipulation to
30   generate human antibodies or analogs with these
characteristics using standard recombinant techniques.
Various techniques for preparing human antibodies using
transgenic nonhuman animals, for example, transgenic
mice, are well known in the art and described, for

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example, in Fishwild et al., Nature Biotechnology 14:
845-851 (1996); Heijnen et al., Journal of Clinical
Investigation 97: 331-338 (1996); Lonberg et al. Nature
368: 856-859 (1994); Morrison, Nature 368: 812-813

5 (1994); Neuberger, Nature Biotechnology 14: 826 (1996);
Chadd and Chamow, Curr. Opin. Biotechnol. 12(2):188-94
(2001); Russel et al., Infection and Immunity 1820-1826
(2000); Gallo et al., European Journal of Immunology
30:534-540 (2000); Davis et al., Cancer Metastasis Rev.

10 18(4):421-5 (1999); Green, Journal of Immunological
Methods 231:11-23 (1999); Yang et al., Journal of
Leukocyte Biology 66:401-410 (1999); Jakobovits, Advanced
Drug Delivery Reviews 31:33-42 (1998); Green and
Jakobovits, J. Exp. Med. 188(3):483-495 (1998);

15 Jakobovits, Exp. Opin. Invest. Drugs 7(4):607-614 (1998);
Mendez et al., Nature Genetics 15:146-156 (1997);
Jakobovits, Weir's Handbook of Experimental Immunology,
The Integrated Immune System, Vol. IV: 194.1-194.7
(1996), each of which is incorporated herein by

20 reference. Furthermore, various techniques known in the
art for preparation of a human antibody are described in
United States Patent Nos. 6,162,963; 6,150,584;
6,114,598; 6,111,166; 6,096,311 and 6,075,181, each of
which is incorporated herein by reference.

25   Another highly efficient means for generating
recombinant antibodies is by primatization as described
by Newman, Biotechnology 10:1455-1460 (1992), which is
incorporated herein by reference. More particularly,
primatized antibodies can be generated which contain

30 monkey variable domains and human constant sequences.
Methods for primatization are known in the art and
described in United States Patent No.6,113,898, which is
incorporated herein by reference. Antibodies can be
primatized such that they are not antigenically rejected

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upon human administration. Primatization relies on
immunization of primates, for example, cynomolgus monkeys
(*Macaca fascicularis*), with human antigens or receptors
and can be used to create high affinity monoclonal
5 antibodies directed to human cell surface antigens.
Antibodies generated by primatization have previously
been reported to display human effector function, have
reduced immunogenicity, and long serum half-life and are
thus useful as neutralizing agents of the invention. The
10 technology relies on the fact that despite the fact that
cynomolgus monkeys are phylogenetically similar to
humans, they still recognize many human proteins as
foreign and therefore mount an immune response.
Moreover, because the cynomolgus monkeys are
15 phylogenetically close to humans, the antibodies
generated in these monkeys have been discovered to have a
high degree of amino acid homology to those produced in
humans. Indeed, after sequencing macaque immunoglobulin
light and heavy chain variable region genes, it was found
20 that the sequence of each gene family was 85-98%
homologous to its human counterpart (Newman et al, supra,
1992). The first antibody generated by primatization, an
anti-CD4 antibody, was 91-92% homologous to the consensus
sequence of human immunoglobulin framework regions
25 (Newman et al, supra, 1992). Methods known in the art
for generation of a primatized antibody and useful for
preparing a neutralizing agent of the invention further
are described by Newman et al., Clinical Immunology
98(2):164-74(2001); and Reddy et al., J Immunol.
30 164(4):1925-33 (2000), both of which are incorporated
herein.

        As described above, antibody neutralizing
agents of the invention include, for example, polyclonal
antibodies, monoclonal antibodies as well as recombinant

versions and functional fragments thereof. Recombinant
versions of antibody neutralizing agents include a wide
variety of constructions ranging from simple expression
and co-assembly of encoding heavy and light chain cDNAs

5   to speciality constructs termed designer antibodies.
Recombinant methodologies, combined with the extensive
characterization of polypeptides within the
immunoglobulin superfamily, and particularly antibodies,
provides the ability to design and construct a vast

10   number of different types, styles and specificities of
binding molecules derived from immunoglobulin variable
and constant region binding domains. Specific examples
include chimeric antibodies, where the constant region of
one antibody is substituted with that of another

15   antibody, and humanized antibodies, described above,
where the complementarity determining regions (CDR) from
one antibody are substituted with those from another
antibody.

     Other recombinant versions of antibody

20   neutralizing agents include, for example, functional
antibody variants where the variable region binding
domain or functional fragments responsible for
maintaining antigen binding is fused to an F_c receptor
binding domain from the antibody constant region. Such

25   variants are essentially truncated forms of antibodies
that remove regions non-essential for antigen and F_c
receptor binding. Truncated variants can be have single
valency, for example, or alternatively be constructed
with multiple valencies depending on the application and

30   need of the user. Additionally, linkers or spacers can
be inserted between the antigen and F_c receptor binding
domains to optimize binding activity as well as contain
additional functional domains fused or attached to effect
biological functions other than IP-10 or Mig

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neutralization. Those skilled in the art will know how
to construct recombinant antibody neutralizing agents
specific for IP-10 or Mig in light of the art knowledge
regarding antibody engineering and given the guidance and
5   teachings herein. A description of recombinant
antibodies, functional fragments and variants and
antibody-like molecules can be found, for example, in
"Antibody Engineering," 2nd Edition, (Carl A.K.
Borrebaeck, Ed.) Oxford University Press, New
10   York, (1995).

         Additional functional variants of antibodies
that can be used as antibody neutralizing agents include
antibody-like molecules other than antigen binding-F_c
receptor binding domain fusions. For example,
15   antibodies, functional fragments and fusions thereof
containing a F_c receptor binding domain can be produced to
be bispecific in that one variable region binding domain
exhibits binding activity for one antigen and the other
variable region binding domain exhibits binding activity
20   for a second antigen. Such bispecific antibody
neutralizing agents can be advantageous in the methods of
the invention because a single bispecific antibody will
contain two different target antigen binding species.
Therefore, a single molecular entity can be administered
25   to achieve neutralization of, for example, both IP-10 and
Mig.

         An antibody neutralizing agent specific for IP-
10 or Mig can also be an immunoadhesion or bispecific
immunoadhesion. Immunoadhesions are antibody-like
30   molecules that combine the binding domain of a non-
antibody polypeptide with the effector functions of an
antibody of an antibody constant domain. The binding
domain of the non-antibody polypeptide can be, for

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example, a ligand or a cell surface receptor having
ligand binding activity. Immunoadhesions for use as IP-
10 or Mig neutralizing agents can contain at least the F_c
receptor binding effector functions of the antibody

5   constant domain. Specific examples of ligands and cell
surface receptors that can be used for the antigen
binding domain of an immunoadhesion neutralizing agent
include, for example, a T cell or NK cell receptor such
as the CXCR3 receptor that recognizes IP-10 and Mig.

10   Other ligands and ligand receptors known in the art can
similarly be used for the antigen binding domain of an
immunoadhesion neutralizing agent specific for IP-10 or
Mig. In addition, multivalent and multispecific
immunoadhesions can be constructed for use as IP-10 and

15   Mig neutralizing agents. The construction of bispecific
antibodies, immunoadhesions, bispecific immunoadhesions
and other heteromultimeric polypeptides which can be used
as IP-10 or Mig specific neutralizing agents is the
subject matter of, for example, U.S. Patent Numbers

20   5,807,706 and 5,428,130, which are incorporated herein by
reference.

In one embodiment of the invention, the
polynucleotides encoding IP-10, Mig, regulatory molecules
that modulate the expression or activity of IP-10 or Mig,

25   or any fragment thereof, or antisense molecules, can be
used as neutralizing agents for therapeutic purposes. In
one aspect, antisense molecules to the IP-10 encoding
nucleic acids can be used to block the transcription or
translation of the mRNA. Specifically, cells can be

30   transformed with sequences complementary to IP-10 nucleic
acids. Such methods are well known in the art, and sense
or antisense oligonucleotides or larger fragments, can be
designed from various locations along the coding or
control regions of sequences encoding IP-10. Thus,

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antisense molecules may be used to neutralize IP-10 or
Mig activity, or to achieve regulation of gene function.

The activity of IP-10 and Mig as well as the
activity of a neutralizing agent can be confirmed by *in*
5 *vitro* assays known in the art. Thus, the invention
further provides a method of identifying a neutralizing
agent specific for IP-10 comprising contacting cells
containing an IP-10 receptor receptor with IP-10 in the
presence of a candidate neutralizing agent, and
10 determining the occurrence of a biological response
associated with IP-10 activity, wherein non-occurrence of
the biological response identifies the candidate as an
IP-10 neutralizing agent. The invention further provides
a method of identifying a neutralizing agent specific for
15 Mig comprising contacting cells containing a Mig receptor
with Mig in the presence of a candidate neutralizing
agent, and determining the occurrence of a biological
response associated with Mig activity, wherein lack of
the biological response identifies the candidate as a Mig
20 neutralizing agent.

As described in Example III, a chemotaxis assay
can be used to evaluate the ability of defined signals to
induce directional migration of a targeted cell
population. Briefly, the activity of IP-10 or Mig can be
25 neutralized with a neutralizing agent of the invention,
for example, a human monoclonal antibody and the
inhibitory effect of this treatment on T cell migration
into the central nervous system can be confirmed. As
further described in Example III, an assay that measures
30 intracellular calcium changes also is useful to confirm
the activity of a neutralizing agent of the invention.
In this regard, a hallmark of chemokine binding to a
specific chemokine receptor is a change in intracellular

calcium levels. Therefore, the ability of a neutralizing
agent to inhibit the interaction of IP-10 or Mig with the
CXCR3 receptor can be confirmed by measuring and
comparing intracellular calcium changes in the absence

5   versus the presence of a neutralizing agent of the
invention. Other assays for identifying a neutralizing
agent specific for IP-10 or Mig that are known in the art
aslo can be used to practice the invention.

Expression vectors derived from retroviruses,

10   adenovirus, adeno-associated virus (AAV), herpes or
vaccinia viruses, or from various bacterial plasmids can
be used for delivery of antisense nucleotide sequences.
The viral vector selected should be able to infect the
central nervous system cells and be safe to the host and

15   cause minimal cell transformation. Retroviral vectors
and adenoviruses offer an efficient, useful, and
presently the best-characterized means of introducing and
expressing foreign nucleotide sequences efficiently in
mammalian cells. These vectors are well known in the art

20   and have very broad host and cell type ranges, express
genes stably and efficiently. Methods well known to
those skilled in the art can be used to construct such
recombinant vectors and are described in Sambrook et al.,
Molecular Cloning: A Laboratory Manual, 2nd ed., Cold

25   Spring Harbor Press, Plainview, New York (1989), and
Ausubel et al., Current Protocols in Molecular Biology,
John Wiley & Sons, New York (1999). Even in the absence
of integration into the DNA, such vectors can continue to
transcribe RNA molecules until they are disabled by

30   endogenous nucleases. Transient expression can last for
a month or more with a non-replicating vector and even
longer if appropriate replication elements are part of
the vector system.

Ribozymes, enzymatic RNA molecules, can also be
used to catalyze the specific cleavage of IP-10 mRNA, Mig
mRNA or the mRNA of any regulatory molecule that
modulates the expression or activity of IP-10 or Mig.
5   The mechanism of ribozyme action involves
sequence-specific hybridization of the ribozyme molecule
to complementary target the mRNA, followed by
endonucleolytic cleavage. Specific ribozyme cleavage
sites within any potential RNA target are identified by
10   scanning the RNA for ribozyme cleavage sites which
include the following sequences: GUA, GUU, and GUC. Once
identified, short RNA sequences of between 15 and 20
ribonucleotides corresponding to the region of the target
gene containing the cleavage site can be evaluated for
15   secondary structural features which can render the
oligonucleotide inoperable. The suitability of candidate
targets can also be evaluated by testing accessibility to
hybridization with complementary oligonucleotides using
ribonuclease protection assays. Antisense molecules and
20   ribozymes of the invention can be prepared by any method
known in the art for the synthesis of nucleic acid
molecules.

The neutralizing agents useful for practicing
the methods of the invention can be formulated and
25   administered by those skilled in the art in a manner and
in an amount appropriate for the demyelinating disease to
be treated; the rate or amount of demyelination; the
weight, gender, age and health of the subject; the
biochemical nature, bioactivity, bioavailability and side
30   effects of the particular compound; and in a manner
compatible with concurrent treatment regimens. An
appropriate amount and formulation for decreasing the
severity of a demyelinating disease in humans can be
extrapolated from credible animal models known in the art

of the particular disorder. It is understood, that the
dosage of a neutralizing agent specific for IP-10 or Mig
has to be adjusted based on the binding affinity of the
neutralizing agent for IP-10 or Mig, such that a lower

5 dose of a neutralizing agent exhibiting significantly
higher binding affinity can be administered compared to
the dosage necessary for a neutralizing agent with lower
binding affinity.

The total amount of neutralizing agent can be

10 administered as a single dose or by infusion over a
relatively short period of time, or can be administered
in multiple doses administered over a more prolonged
period of time. Such considerations will depend on a
variety of case-specific factors such as, for example,

15 whether the disease category is characterized by acute
episodes or gradual neurologic deterioration. For
example, for a subject affected with chronic neurologic
deterioration the neutralizing agent can be administered
in a slow-release matrice, which can be implanted for

20 systemic delivery or at the site of the target tissue.
Contemplated matrices useful for controlled release of
therapeutic compounds are well known in the art, and
include materials such as DepoFoamTM, biopolymers,
micropumps, and the like.

25 The neutralizing agents of the invention can be
administered to the subject by any number of routes known
in the art including, for example, systemically, such as
intravenously or intraarterially. An IP-10 or Mig
specific neutralizing agent can be provided in the form

30 of isolated and substantially purified polypetides and
polypeptide fragments in pharmaceutically acceptable
formulations using formulation methods known to those of

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ordinary skill in the art. These formulations can be
administered by standard routes, including for example,
topical, transdermal, intraperitoneal, intracranial,
intracerebroventricular, intracerebral, intravaginal,
5   intrauterine, oral, rectal or parenteral (e.g.,
intravenous, intraspinal, intrathecal, subcutaneous or
intramuscular) routes. Intrathecal administration of an
IP-10 or Mig specific neutralizing agent into the
intradural or subarachnoid space is a preferred route for
10   practicing the methods of the invention. Intravenous
administration of an IP-10 or Mig specific neutralizing
agent also is a preferred route for practicing the
methods of the invention. In addition, an IP-10 or Mig
specific neutralizing agent variant can be incorporated
15   into biodegradable polymers allowing for sustained
release of the compound useful for reducing the severity
of a demyelinating disease. Biodegradable polymers and
their use are described, for example, in Brem et al., J.
Neurosurg. 74:441-446 (1991), which is incorporated
20   herein by reference.

An IP-10 or Mig specific neutralizing agent can
be administered as a solution or suspension together with
a pharmaceutically acceptable medium. Such a
pharmaceutically acceptable medium can be, for example,
25   sterile aqueous solvents such as sodium phosphate buffer,
phosphate buffered saline, normal saline or Ringer's
solution or other physiologically buffered saline, or
other solvent or vehicle such as a glycol, glycerol, an
oil such as olive oil or an injectable organic ester. A
30   pharmaceutically acceptable medium can additionally
contain physiologically acceptable compounds that act,
for example, stabilize the neutralizing agent, increase
its solubility, or increase its absorption. Such
physiologically acceptable compounds include, for

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example, carbohydrates such as glucose, sucrose or
dextrans; antioxidants such as ascorbic acid or
glutathione; receptor mediated permeabilizers, which can
be used to increase permeability of the blood-brain

5   barrier; chelating agents such as EDTA, which disrupts
microbial membranes; divalent metal ions such as calcium
or magnesium; low molecular weight proteins; lipids or
liposomes; or other stabilizers or excipients. Those
skilled in the art understand that the choice of a

10   pharmaceutically acceptable carrier depends on the route
of administration of the compound containing the
neutralizing agent and on its particular physical and
chemical characteristics.

             Formulations suitable for parenteral
15   administration include aqueous and non-aqueous sterile
injection solutions such as the pharmaceutically
acceptable mediums described above. The solutions can
additionally contain, for example, buffers, bacteriostats
and solutes which render the formulation isotonic with

20   the blood of the intended recipient. Other formulations
include, for example, aqueous and non-aqueous sterile
suspensions which can include suspending agents and
thickening agents. The formulations can be presented in
unit-dose or multi-dose containers, for example, sealed

25   ampules and vials, and can be stored in a lyophilized
condition requiring, for example, the addition of the
sterile liquid carrier, immediately prior to use.
Extemporaneous injection solutions and suspensions can be
prepared from sterile powders, granules and tablets of

30   the kind previously described.

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For applications that require the compounds and compositions to cross the blood-brain barrier, formulations that increase the lipophilicity of the compound are particularly desirable. For example, the

5   neutralizing agent can be incorporated into liposomes (Gregoriadis, Liposome Technology, Vols. I to III, 2nd ed. (CRC Press, Boca Raton FL (1993)). Liposomes, which consist of phospholipids or other lipids, are nontoxic, physiologically acceptable and metabolizable carriers

10 that are relatively simple to make and administer.

A neutralizing agent specific for IP-10 or Mig can also be prepared as nanoparticles. Adsorbing peptide compounds onto the surface of nanoparticles has proven effective in delivering peptide drugs to the brain (see

15 Kreuter et al., Brain Res. 674:171-174 (1995)). Exemplary nanoparticles are colloidal polymer particles of poly-butylcyanoacrylate with a neutralizing agent specific for IP-10 or Mig adsorbed onto the surface and then coated with polysorbate 80.

20   Image-guided ultrasound delivery of a neutralizing agent through the blood-brain barrier to selected locations in the brain can be utilized as described in U.S. Patent No. 5,752,515. Briefly, to deliver a neutralizing agent past the blood-brain barrier

25 a selected location in the brain is targeted and ultrasound used to induce a change detectable by imaging in the central nervous system (CNS) tissues and/or fluids at that location. At least a portion of the brain in the vicinity of the selected location is imaged, for example,

30 via magnetic resonance imaging (MRI), to confirm the location of the change. An IP-10 or Mig specific neutralizing in the patient's bloodstream can delivered

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to the confirmed location by applying ultrasound to
effect opening of the blood-brain barrier at that
location and, thereby, to induce uptake of the
neutralizing agent.

5   In addition, polypeptides called receptor
mediated permeabilizers (RMP) can be used to increase the
permeability of the blood-brain barrier to molecules such
as therapeutic agents or diagnostic agents as described
in U.S. Patent Nos. 5,268,164; 5,506,206; and 5,686,416.
10   These receptor mediated permeabilizers can be
intravenously co-administered to a host with molecules
whose desired destination is the cerebrospinal fluid
compartment of the brain. The permeabilizer polypeptides
or conformational analogues thereof allow therapeutic
15   agents to penetrate the blood-brain barrier and arrive at
their target destination.

          In current treatment regimes for demyelinating
diseases, more than one compound is often administered to
an individual for management of the same or different
20   aspects of the disease. Similarly, in the methods of the
invention involving decreasing the rate of or reversing
demyelination, a neutralizing agent specific for IP-10 or
Mig can advantageously be formulated with a second
therapeutic compound such as an anti-inflammatory
25   compound, immunosuppressive compound or any other
compound that manages the same or different aspects of
the disease. Such compounds include, for example,
methylprednisolone acetate, dexamethasone and
betamethasone. Contemplated methods of reducing the
30   severity of a demyelinating disease and promoting
remyelination include administering a neutralizing agent
specific for IP-10 or Mig alone, in combination with, or

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in sequence with, such other compounds. Alternatively,
combination therapies can consist of fusion proteins,
where the neutralizing agent specific for IP-10 or Mig is
linked to a heterologous protein, such as a therapeutic

5   protein.

        The following examples are intended to
illustrate but not limit the present invention.

        It is understood that modifications which do
not substantially affect the activity of the various

10 embodiments of this invention are also included within
the definition of the invention provided herein.
Accordingly, the following examples are intended to
illustrate but not limit the present invention.

                        EXAMPLE I

15   __Selective Inhibition of IP-10 with Anti-IP-10 Antibody__

        This example describes selective inhibition of
IP-10 in mice with a rabbit polyclonal anti-IP-10
antibody, which resulted in increased mortality and
decreased T lymphocyte infiltration of the CNS in

20 response to viral infection. In addition, the selective
inhibition of IP-10 in mice following viral infection of
the CNS resulted in a reduction in demyelination of nerve
fibers that was accompanied by a gross increase in
remyelination.

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In order to determine the functional
significance of IP-10 expression following viral
infection of the CNS, mice were infected with a
neurotropic strain of MHV, a positive-strand RNA virus.
5   Intracranial infection of susceptible mice with MHV
results in a chronic demyelinating disease that shares
many clinical and histologic similarities with MS. As
such, the MHV mouse model is a well accepted animal model
for studying the underlying immunopathological mechanisms
10   contributing to the pathogenesis of MS. Specifically,
intracranial infection of mice with MHV results in an
acute encephalomyelitis followed by chronic neurological
disease in susceptible strains of mice (see Houtman and
Fleming, J. Neurovirol. 2:361-376(1996); Buchmeier and
15   Lane, Curr. Opin. Micro. 2:398-402 (1999)). The acute
stage of MHV is represented by widespread viral infection
of neurons and glial cells, whereas the chronic stage is
characterized by viral persistence in astrocytes and
oligodendrocytes accompanied by mononuclear cell
20   infiltration and myelin destruction (see Houtman and
Fleming, supra, 1996; Buchmeier and Lane, supra, 1999).

The Mouse Hepatitis Virus (MHV) strain V5A13.1
was derived from wild-type MHV-4 as described by Dalziel
et al., J. Virol. 59: 463-471 (1986), which is
25   incorporated herein by reference. Age matched five to
seven week old male wt C57BL/6 (H-2b background) mice
(Sprague-Dawley, San Diego, CA) were used for the
described studies. Following anesthetization by
inhalation of methoxyfluorane (Pitman-Moore Inc.,
30   Washington Crossing, NJ), the mice were injected
intracranially with 10pfu of MHV suspended in 30µl of
sterile saline as described in Lane et al., J. Virol.
74:1415-1424 (2000), which is incorporated herein by

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reference. Control animals were injected with sterile
saline alone. The animals were sacrificed at days 7 and
10 post infection, at which time brains and spinal cords
were removed. One-half of each brain was used for the
5   plaque assay on the DBT astrocytoma cell line to
determine viral burden as described in Lane et al.,
supra, 2000. The remaining half of each brain was either
fixed for histological analysis, stored at -80°C for RNA
isolation, or used for FACS analysis or ELISA. All data
10   were analyzed by performing the Mann-Whitney Rank Sum
test using Sigma Stat 2.0 software, with P values of 0.05
or smaller considered significant.

IP-10 is expressed very early (day 1 post
infection) within the CNS following MHV infection and
15   remains the predominant chemokine expressed during the
acute phase of the disease (see Lane et al., J. Immunol.
160:970-978 (1998)). IP-10 activity was selectively
inhibited through intraperitoneal administration of
rabbit polyclonal anti-IP-10 antisera.

20   The rabbit polyclonal antisera specific for
mouse IP-10 was generated as described in Tannenbaum et
al., J. Immunol. 161: 927-932 (1998), which is
incorporated herein by reference. The rabbit polyclonal
antibody has been shown previously to be specific for IP-
25   10 and not cross-react with other known chemokines (see
Tannenbaum et al., supra, 1998). MHV-infected mice were
divided into two groups and treated with either normal
rabbit serum (NRS) or anti-IP-10. Mice were injected
intraperitoneally with 0.5 ml of anti-IP-10 antisera at a
30   concentration of 0.5 mg/ml or with NRS on days 0, 2, 5,
7, and 9 post infection and subsequently sacrificed at
days 7 and 10 post infection.

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Treatment with rabbit polyclonal antiserum
specific for mouse IP-10 led to an increase in mortality
with less than 5 percent of any anti-IP-10 mice surviving
until day 12 post infection (n=27)(Figure 1). In
5   contrast, approximately 50 percent of NRS treated control
mice survived MHV infection(n=27) (Figure 1).
Correlating with increased mortality was a decrease in
the ability of anti-IP-10-treated mice to clear virus
from the CNS as compared to NRS-treated mice.
10 Specifically, surviving anti-IP-10 treated mice displayed
a 2-log increase in viral titers in the brain as compared
to titers present in NRS-treated mice at day 10 post
infection (Figure 2).

Previous studies have shown that CD4$^+$ and CD8$^+$
15 cells are important in clearing MHV from the CNS (see
Williamson and Stohlman, J. Virol. 64:4589-4592 (1990);
Yamaguchi et al., J. Neuroimmunol. 32:1-9 (1991); Pearce
et al., J. Virol. 68:5483-5495 (1997); Lane et al.,
supra, 2000). In this study, cells were obtained from
20 brains of anti-IP-10 or NRS treated MHV-infected mice at
7 days post infection and dual fluorescent staining was
performed for CXCR3 and CD4 or CD8 antigen as preciously
described in Lane et al., supra, 2000. Fluorescein
isothiocyanate-conjugated (FITC) rat anti-mouse antibody
25 was used to detect CD4 and CD8 positive T cells
(Pharmingen, San Diego, CA). As a control, an isotype-
matched FITC-conjugated antibody was used. Cells were
incubated with antibodies for 30 minutes at 4°C, washed
and subsequently fixed in 1 percent paraformaldehyde and
30 finally analyzed on a FACStar (Becton Dickinson, Mountain
View, CA).

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Dual fluorescent staining CD4$^+$ and CD8$^+$ lymphocytes as well as confocal microscopy showed that the majority of CD4$^+$ and CD8$^+$ lymphocytes that infiltrate the CNS in response to viral infection express the IP-10 receptor CXCR3. CD4$^+$ and CD8$^+$ T lymphocytes expressing CXCR3 were present within the meninges as well as the parenchyma indicating these cells were able to migrate into the brain. Overall, these results demonstrate that IP-10 is a modulator of the host defense by attracting T cells into the CNS in response to viral infection. Furthermore, as shown in Figure 3, flow-cytometric analysis revealed that anti-IP-10 treatment of mice resulted in a 82.3 percent decrease in infiltrating CD4+ T cells and a 70.4 percent decrease in infiltrating CD8+ cells compared to NRS treatment of mice. Both, anti-IP-10 treated and NRS treated mice displayed comparable levels of monocyte/macrophage infiltration, indicating that IP-10 does not attract these cells into the CNS following viral infection.

The number of T lymphocytes infiltrating the CNS in anti-IP-10 treated mice and the CXCR3 expression on infiltrating CD4$^+$ and CD8$^+$ T lymphocytes was determined with primary antibodies diluted in buffered saline containing 5 percent normal horse serum used for dual fluorescent detection of cellular antigens as follows: Rat anti-mouse CD4 (Pharmingen, San Diego, CA) at a dilution of 1:100; and goat anti-mouse CXCR3 (Santa Cruz Laboratories, Santa Cruz, CA) at a dilution of 1:50. For CD4 and CD8 primary antibodies, a TRITC-conjugated secondary antibody was used at a dilution of 1:50 (Sigma, ST. Louis, MO). For CXCR3 primary antibody, an FITC conjugated secondary antibody was used at a dilution of 1:50 (Zymed, South San Francisco, CA). The reagent used to detect F4/80, an antigen specific to macrophages, is a

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monoclonal antibody (clone C1:A3-1) ·(Serotec, Raleigh,
NC) which was used at a 1:50 dilution in a 2% goat
serum/PBS solution according to the manufacturer's
instructions. Staining was performed on 8µm frozen

5   sections fixed in acetone for 10 minutes at -20°C.
Subsequently, dual stained slides were subjected to
confocal microscopy using a BioRad MRC UV laser scanning
confocal microscope.

To determine whether infiltrating T lymphocytes

10   contribute to the host defense against MHV infection of
the CNS through the release of the anti-viral cytokine
IFN-γ, levels of mRNA and protein within the brains of
anti-IP-10 and NRS treated mice were determined by
ribonuclease protection assay (RPA) and ELISA,

15   respectively.

Total RNA was extracted from the brains of NRS
treated or anti-IP-10 treated mice at day 7 post
infection using TRIzol® (Life Technologies (GIBCO-BRL)
Rockville, MD) reagent as described in Lane et al.,

20   supra, 2000; and Lane et al., J. Immunol., 160:970-978
(1998). The ribonuclease protection assay was performed
using 15 µg of total RNA as previously described in Lane
et al., supra, 2000; and Lane et al., supra, 1998.
Interferon-γ (IFN-γ) levels were quantified using the

25   Quantikine M mouse IFN-γ immunoassay kit (R&D Systems,
Minneapolis, MN) using brains of mice obtained at day 7
post infection as described in Lane et al., supra, 2000.
The data shown in Figure 4A is presented as normalized
units representing the ratio of signal intensity of IFN-γ

30   to internal L32 included in the probe set. Analysis of
the values obtained from the scanned autoradiograph was
performed using NIH Image 1.61 software.

As shown in Figure 4A, the neutralization of
IP-10 resulted in decreased mRNA transcripts for IFN-γ as
compared to transcript levels present in the brains of
NRS-treated mice. Correlating with the decrease in IFN-γ
5   transcript levels was an approximate 80 percent decrease
in IFN-γ protein levels at day 7 post infection compared
to protein levels in NRS treated mice (Figure 4B).
Although the levels of IFN-γ mRNA transcripts in anti-
IP10 treated mice were slightly higher than would be
10   predicted based on the IFN-γ ELISA data, this is most
likely due to mouse-to-mouse variation and sensitivity in
the RPA and not the result of IP-10 modulating IFN-γ mRNA
translation.

To determine the effect of a continuous course
15   of anti-IP-10 and anti-Mig treatment on MHV infected
mice, treatment was administered continuously from 12
days until 19 days post infection. Figure 5 shows the
clinical score of MHV infected mice treated with either
anti-IP-10, anti-Mig or NRS starting at 12 days and
20   ending at 19 days post infection. The clinical score for
the three populations was substantially identical until
the start of treatment and remained substantially
identical for mice treated with anti-Mig and those
treated with NRS (Figure 5). However, the clinical score
25   for anti-IP-10 treated mice significantly declined
compared to the anti-Mig and NRS treated mice during
treatment and recovered once treatment was discontinued
(Figure 5).

Post treatment infiltration of CD4 and CD8 T
30   lymphocytes into the CNS of mice treated with anti-IP-10,
anti-Mig or NRS was measured at 28 days post infection by
FACS analysis as described above. As shown in Figure 6,

anti-IP-10 treatment of MHV infected mice starting at day
12 and ending at day 19 post infection resulted in no
significant differences in CD4$^+$ or CD8$^+$ infiltration into
the CNS compared to NRS treatment (Figure 6).

5   Post treatment CNS infiltration of CD4$^+$ T
lymphocytes, CD8$^+$ T lymphocytes and macrophages was
measured at day 21 post infection in mice treated with
anti-IP-10, anti-Mig or NRS starting at day 12 post
infection and ending at day 19 post infection via FACS
10   analysis as described above. As shown in Figure 7, anti-
IP-10 treatment of MHV infected mice starting at day 12
and ending at day 19 post infection resulted in a
significant decrease in infiltration of macrophages into
the CNS at 21 days post infection compared to NRS
15   treatment. Similarly, anti-IP-10 treatment of MHV
infected mice starting at day 12 and ending at day 19
post infection resulted in a significant decrease in
infiltration of CD4$^+$ cells into the CNS at 21 days post-
infection compared to NRS treatment. Macrophages are
20   designated F480 in Figure 7, based on the antigenic
marker F4/80 used for their detection.

          Demyelination of CNS nerve fibers in MHV
infected mice treated with anti-IP-10, anti-Mig or NRS
starting at day 12 and ending at day 19 post infection
25   was measured at 21 days post infection. Demyelination
was assessed by examination of spinal cords from animals
within selected experimental groups. Tissue was prepared
and the severity of demyelination was assessed as
follows: Spinal cords were removed from mice at
30   scheduled time points and fixed overnight in 10% normal
buffered formalin. Tissues were then embedded in
paraffin. Subsequently, sections were stained with both

hematoxylin and eosin to detect inflammation and luxol fast blue to detect areas of demyelination. Following the staining, the slides were blinded and independently read by three investigators. Scoring was based on a

5   recently described scale (Houtman and Fleming, supra, 1996). A score of 0 indicated normal tissue; 1 indicated mild inflammation with limited white matter destruction; 2 represented a moderate increase in both inflammation and demyelination; 3 represented a

10 pronounced increase in both inflammatory lesions and demyelination; and 4 indicated numerous inflammatory/demyelinating lesions. Scores were averaged between the three investigators and presented as the mean + standard error of the mean (SEM).

15   As shown in Figure 8, anti-IP-10 treatment from day 12 until day 19 post infection resulted in a significant reduction in demyelination at day 21 post infection compared to NRS treatment. This reduction in demyelination corresponds to a reduction in F480 cell

20 infiltration and CD4 cell infiltration into the CNS of MHV infected mice treated with anti-IP-10 from day 12 until day 19 post infection at 21 days post infection compared to NRS treatment.

          To determine whether the significant reduction

25 in demyelination detected in mice treated with IP-10 was accompanied by a gross increase in remylination light microscopic analysis of spinal cord sections of anti-IP-10, anti-Mig and NRS treated MHV infected mice was performed. Remyelination was assessed by light

30 microscopic analysis of toluene blue stained spinal cord sections (coronal) obtained at day 21 post-infection. The significant reduction in demyelination detected in

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mice treated with anti-IP-10 at day 21 post-infection
described above (see Figure 8) was accompanied by a gross
increase in remyelination. Neither mice treated with NRS
or anti-Mig experienced remyelination. The results of

5   the light microscopic analysis indicate that the
anti-IP-10 mediated neutralization of CD4 and macrophage
entry into the CNS not only prevents further
demyelination, but also allows repair of the damaged
myelin. Figure 16 shows electron photomicrographs of

10 axons corresponding to MHV infected mice. As shown in
Figure 16A, anti-IP-10 treatment led to remyelination of
viral induced demyelination as indicated by the
characteristically thin dark lines surrounding each of
the axons. In contrast, Figure 16B shows untreated axons

15 with no surrounding myelin as a result of viral-induced
demyelination.

EXAMPLE II

Selective Inhibition of Mig with Anti-Mig Antibody

     This example shows selective inhibition of Mig

20 in mice with an anti-Mig polyclonal antibody, which
resulted in increased mortality during the acute phase of
MHV infection, correlating with an increased viral burden
on the CNS. In addition, the results described below
indicate that Mig is important during the acute phase of

25 the disease, but does not contribute to the pathology of
chronic demyelination. Furthermore, this example shows
that antibody mediated neutralization of Mig activity
results in a shift from a protective Th1 to a Th2
response. This shift in host response is characterized

30 by a significant reduction in CD4+ and CD8+ T cell
infiltration into the CNS, decreased IFN-γ expression and

increased levels of the anti-inflammatory Th2 cytokine
IL-10 compared to levels found in NRS treated mice.

       The mice were infected with MHV and the brains
and spinal cords were isolated as described above.
5   Rabbit polyclonal anti-Mig antibody was produced by
Biosynthesis (Lewisville, TX) using a synthetic peptide
CISTSRGTIHYKSLK, coupled to carrier protein KLH selected
from the Mig protein sequence as described in Tannenbaum
et al., *supra*, 1998. These reagents have previously been
10   shown to be specific to Mig and not cross react with IP-
10, RANTES, or other chemokines (see Tannenbaum et al.,
supra, 1998). MHV-infected mice were divided into two
groups and treated with either NRS or anti-Mig. Mice
were injected with 0.5 ml of antibody at a concentration
15   of 0.5 mg per ml on days 0, 2, 5, 7, and 9 post infection
and sacrificed at days 7 and 10 post infection.

       As shown in Figure 9, surviving anti-Mig
treated mice displayed a 2-log increase in viral titers
in the brain as compared to titers present in NRS-treated
20   mice at day 10 post infection, which is similar to the
kinetics of viral clearance shown for anti-IP-10
treatment in Figure 2 and described above. During the
acute stage of the disease which lasts until day 7 post
infection, virus replicates within neurons as well as
25   glial cells (see Houtman and Fleming, supra, 1996;
Dalziel, supra, 1985; and Fazackerly et al, Virology
187:178-188 (1992)). Animals that survive acute disease
often develop a chronic demyelinating disease
characterized by viral persistence within astrocytes and
30   oligodendrocytes accompanied by mononuclear cell
infiltration and myelin destruction (see Houtman and
Fleming, supra, 1996; Buchmeier and Lane, supra, 1999).

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To determine the role of Mig in the course of
the MHV infection, total RNA was isolated from brains at
days 7, 12 and 35 post infection and the kinetics of Mig
and MHV viral gene expression were determined by
5   ribonuclease protection assay (RPA). Total RNA was
extracted from brains and spinal cords of MHV-infected
animals treated with either anti-Mig or NRS as described
in Lane et al., *supra*, 1998. The antisense riboprobe
used to detect Mig mRNA was derived by RT-PCR
10   amplification of cDNA generated from total RNA isolated
from the brain of an MHV-infected mouse at day 7, 12 and
35 post infection. The following oligonucleotide primers
were used for Mig amplification: (Forward) 5' CGT CGT CGT
TCA AGG AAG and (Reverse) 5' TCG AAA GCT TGG GAGGTT. PCR
15   amplification was performed using an automated Perkin-
Elmer (Norwalk, CT) model 480 DNA thermocycler with the
following profile: step 1, initial denaturation at 94°C
for 30 seconds; step 2, annealing at 60°C for 30 seconds;
and step 3, extension at 72°C for 45 seconds. Steps 1
20   through 3 were repeated 34 times for a total of 35 cycles
and followed by a 7 minute incubation at 72°C. The
expected PCR amplicon of 599 base pairs was cloned into
the pCR Script SK+ vector (Stratagene, San Diego, CA) and
sequence analysis identified over 95 percent nucleotide
25   identity with mouse Mig (see Vanguri and Farber, J. Biol.
Chem. 265(25):15049-15057(1990).

For analysis of Mig and MHV viral gene
expression, the Mig riboprobe was used in combination
with a riboprobe specific for the MHV spikegene
30   previously described in Lane et al., J. Virol. 71:2202-
2210 (1997), which is incorporated herein by reference.
L32 was added as an internal control to verify
consistency in RNA loading and assay performance as

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previously described in Lane et al., _supra_, 1998; and
Lane et al., _supra_, 2000. For quantification of signal
intensity, autoradiographs were scanned and individual
chemokine bands were normalized as the ratio of band

5   intensity to the L32 control as previously described in
Lane et al., _supra_, 1998; and Lane et al., _supra_, 2000.
Analysis of the values obtained from the scanned
autioradiograph was performed using NIH Image 1.61
software.

10   As shown in Figure 10, the Mig and MHV spike
genes are expressed at days 7 and 12 post infection.
However, by day 35 post infection, a time at which the
animals exhibit extensive myelin loss (see Figure 8), Mig
transcripts are undetectable whereas spike gene

15   transcripts are still present. Double labeling using
antisense riboprobe specific for Mig mRNA and a
polyclonal antibody to glial fibrillary acidic protein
(GFAP) (DAKO Corp., Carpinteria, CA) used according to
manufacturer's instructions, indicate that astrocytes

20   express Mig following MHV infection. These results
indicate that Mig is important during the acute phase of
the disease but does not contribute to chronic
demyelination within persistently infected mice.

          For studies designed to colocalize cellular

25   antigens with in situ signal for Mig mRNA transcripts,
immunohistochemical analysis preceded in situ
hybridization performed as described in Lane et al,
supra, 1998. The phosphate-buffered saline (PBS) used
for dilution of antibodies as well as in washing steps

30   was diethyl pyrocarbonate treated to reduce RNase
contamination and loss of in situ signal. Following
application of the chromagen diaminobenzidine (DAB),

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slides were washed twice in PBS and then prehybridized in
hybridization buffer for 1 hour at 42°C. Subsequently,
the linearized 35S-labeled riboprobe was added to the
sections and the standard in situ hybridization procedure

5 was performed as described in Lane et al., supra, 1998.
Upon development, the slides were counterstained in
hematoxylin only, then dehydrated and mounted.

        Treatment of MHV infected mice with anti-Mig
antibody was performed to evaluate the functional

10 significance of Mig expression following viral infection
of the CNS. As shown in Figure 11, anti-Mig antibody
treatment resulted in a marked increase in mortality as
compared to control mice treated with NRS. By day 12
post infection, less than 10 percent of the anti-Mig

15 treated mice survived the infection in contrast to 50
percent survival rate in MHV infected mice treated with
NRS (Figure 11). Surviving mice treated with anti-Mig
antibody displayed significantly higher ($p \leq$ 0.001) virus
titers (5.4 $\pm$ 0.3, n=4) as compared to titers present in

20 NRS treated mice (2.0 $\pm$ 0.2, n=10) (Figure 9). These
results indicate that neutralization of Mig during the
acute disease results in increased mortality that
correlates with an increase in viral burden within the
CNS.

25   T lymphocyte isolation and flow cytometry was
performed to determine the effect of anti-Mig treatment
of MHV infected mice on T lymphocyte infiltration into
the CNS as described above and in Lane et al., supra,
2000. Fluorescent Activated Cell Sorter (FACS) analysis

30 at day 7 post infection indicated that MHV-infected mice
treated with anti-Mig displayed significantly lower ($p \leq$
0.005) levels of both $CD4^+$ and $CD8^+$ T lymphocyte

infiltration into the CNS when compared to levels present
within MHV infected mice treated with NRS (Figure 12).
As shown in Figure 12, anti-Mig treatment of mice
resulted in a 45 percent reduction in infiltrating CD4$^+$

5   cells and a 60 percent reduction infiltrating CD8$^+$ cells
when compared to NRS treated mice (*p $\leq$ 0.005).

        One mechanism by which T cells contribute to
host defense against MHV infection of the CNS is through
the release of the anti-viral cytokine IFN-γ (Pearce,

10   supra, 1997; Lane, supra, 1997; Parra et al., J. Immunol.
162:1641–1647 (1999).   To determine whether IFN-γ levels
were altered in MHV infected mice treated with anti-Mig,
cytokine mRNA transcript levels within the CNS of ant-Mig
or NRS treated mice were evaluated by RPA at day 7 post

15   infection.

        Figure 13 shows densitometric analysis of IFN-γ
and IFN-β transcript levels in brains of MHV infected
mice treated with either anti-Mig or NRS at day 7 post
infection.   The data shown in Figure 13 is presented as

20   normalized units representing ratio of signal intensity
of either IFN-γ or IFN-β to internal L32 control included
in the probe set.   Values were obtained from the scanned
autoradiograph using NIH Image 1.61 software as described
in Lane et al., supra, 1998 and Lane et al., supra, 2000.

25   The signal intensities indicated that anti-Mig treated
mice displayed an 86.7 percent reduction in IFN-γ (p $\leq$
0.03) as well as a 65.1 percent reduction in IFN-β (p $\leq$
0.02) transcript levels as compared to mice treated with
NRS (Figure 13).

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In addition, IFN-γ and IL-10 protein levels
within groups were determined using the Quantikine M
mouse IFN-γ or IL-10 immunoassay kit (R&D Systems,
Minneapolis, MN) at day 7 post infection. Tissue samples
5   were homogenized in 1 ml of sterile phosphate buffered
saline and spun at 400 x g for 5 minutes at 4°C.
Duplicate supernatant samples were used to determine
respective protein levels present within the tissues
according to the manufacturer's instructions. Following
10 the enzymatic color reaction, samples were read at 450nm
and respective protein levels were quantified in
comparison to a standard curve supplied by the
manufacturer. The results in Figures 14 and 15 are
presented as picograms per milliliter. The limit of
15 sensitivity of protein detection was approximately
8.0pg/ml. The reagents used do not cross react with
other mouse cytokines.

Consistent with the RPA results, IFN-γ protein
levels were reduced by 91 percent ($p \leq 0.05$) within the
20 CNS of anti-Mig treated mice as compared to NRS-treated
mice as determined by ELISA (Figure 14). As shown in
Figure 15, a four-fold increase in levels of the anti-
inflammatory TH2 cytokine IL-10 within the CNS coincided
with decreased IFN-γ levels in mice treated with anti-Mig
25 antibody.

EXAMPLE III

Confirmation of IP-10 And Mig Activity by In-Vitro Assays

This example shows confirmation of IP-10 and
Mig activity by *in vitro* methods as well as confirmation
30 of neutralizing activity of a human monclonal antibody

against IP-10. The assays described below are useful to
identify a neutralizing agent specific for IP-10 or Mig.

        Briefly, the activity of IP-10 or Mig will be
neutralized with a neutralizing agent of the invention,
5   for example, a human monoclonal antibody and the effect
of this treatment on T cell migration into the central
nervous system will be determined. To this end, the
chemotaxis assay will utilize a human cell line that has
been stably transfected with CXCR3 human receptor cDNA
10 and characterized. Briefly, human CXCR3 receptor cDNA
was cloned by RT-PCR from PHA/IL-2 activated human T
cells and RNA prepared. Subcloned cDNA was subsequently
sequenced to confirm PCR primer specificity. Jurkat
cells were electroporated with with 20ug of the cDNA
15 construct. Neomycin-resistant cells were cloned by
limiting dilution and screened by Northern blot for
expression. Subsequently, flow cytometric analysis using
antihuman CXCR3 receptor antibody (Leukocyte, Inc.,
Cambridge, MA) confirmed receptor expression.

20   Chemotaxis will be assessed using a
well-established protocol described in Luster et al.,
Proc. Assoc. Am Physicians 110(3):183-196 (1998), which
is incorporated herein by reference. Briefly, chemotaxis
will utilize 48-well chemotaxis chambers (Neuroprobe,
25 Cabin John, MD) using polyvinylpyrrolidone-free
polycarbonate membranes with 5 micron pores. The
membranes will be submerged in a 2ug/ml solution of human
collagen type IV (Collaborative Biomedical Products,
Beford, MA) and incubated at 37°C for 1 hr prior to use.
30 Human recombinant proinflammatory molecule IP-10
(PeproTech, Rocky Hill, NJ) or Mig will be incubated
either with or without the compounding human mAb and

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receptor-expressing T cells at a concentration of 5x10^4/well and obtained from peripheral bleed followed by Ficoll-Hypaque gradient spin. Activated cells will migrate in response to active proinflammatory molecule
5   and cells will be stained with Diff-Quick (Baster, Valencia, CA) and counted in 4 randomly high-power fields. Non-transfected cells will be used as negative controls.

        A second assay is based on detection of
10 intracellular calcium, changes in which are a hallmark of chemokine binding to a specific chemokine receptor. Intracellular calcium ($[Ca^{2+}]$i) in single, receptor-expressing T cells as described above will be determined using a previously described protocol
15 described in Luster et al., Proc. Assoc. Am Physicians 110(3):183-196 (1998). Cells (1x10^5/ml) will be loaded with fura-2/AM at 2mM at 37°C for 30 min in PIPES buffer. One ml of cells will be in a special chamber (Nunc, Roskilde, Denmark) without any coating of chamber to
20 eliminate background signaling. Changes in $[Ca^{2+}]$i will be determined using a digital imaging system with a light-sensitive camera. Recombinant human proinflammatory molecule will be incubated with or without mAb at different concentration and added to
25 separate chambers of cells. The $[Ca^{2+}]$i will be determined before and after stimulation.

        Throughout this application various publications have been referenced within parentheses. The disclosures of these publications in their entireties
30 are hereby incorporated by reference in this application in order to more fully describe the state of the art to which this invention pertains.

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Although the invention has been described with
reference to the disclosed embodiments, those skilled in
the art will readily appreciate that the specific
experiments detailed are only illustrative of the
5   invention. It should be understood that various
modifications can be made without departing from the
spirit of the invention. Accordingly, the invention is
limited only by the following claims.

What is claimed is:

      1.   A method of promoting remyelination in a
subject affected with a demyelinating disease, comprising
administering to a subject having a demyelinating disease
5   an effective amount of a neutralizing agent specific for
interferon inducible protein of 10 kDa (IP-10).

      2.   The method of claim 1, wherein said subject is
a mammal.

      3.   The method of claim 2, wherein said subject is
10   human.

      4.   The method of claim 1, wherein said
demyelinating disease is multiple sclerosis (MS).

      5.   The method of claim 1, wherein said
neutralizing agent specific for interferon inducible
15   protein of 10 kDa (IP-10) is anti-IP-10 antibody.

      6.   A method of reducing the severity of a
neuroinflammatory disease in a subject, comprising
administering to a subject having a neuroinflammatory
disease an effective amount of a neutralizing agent
20   specific for interferon inducible protein of 10 kDa (IP-
10).

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    7.   A method of reducing the severity of a neuroinflammatory disease in a subject, comprising administering to a subject having a demyelinating disease an effective amount of an antibody specific for

5 interferon inducible protein of 10 kDa (IP-10).

    8.   A method of inhibiting T-cell migration into the central nervous system (CNS) associated with a neuroinflammatory disease, comprising administering to a subject an effective amount of a neutralizing agent

10 specific for interferon inducible protein of 10 kDa (IP-10).

    9.   A method of reducing the severity of a demyelinating disease, comprising remyelinating demyelinated nerve fibers and impeding demyelination of

15 said nerve fibers by administering to a subject having a demyelinating disease an effective amount of a neutralizing agent specific for interferon inducible protein of 10 kDa (IP-10).

    10.   The method of claim 9, wherein said subject is

20 a mammal.

    11.   The method of claim 10, wherein said subject is human.

    12.   The method of claim 9, wherein said demyelinating disease is multiple sclerosis (MS).

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13.   The method of claim 9, wherein said neutralizing agent specific for interferon inducible protein of 10 kDa (IP-10) is anti-IP-10 antibody.

14.   The method of claim 9, wherein said
5   neutralizing agent specific for interferon inducible protein of 10 kDa (IP-10) is administered by intravenous administration.

15.   A method of identifying a neutralizing agent specific for IP-10 comprising contacting cells expressing
10   an IP-10 receptor with IP-10 in the presence of a candidate neutralizing agent, and determining the occurrence of a biological response associated with IP-10 activity, wherein non-occurrence of said biological response identifies said candidate as an IP-10
15   neutralizing agent.

16.   The method of claim 15, wherein said biological response is a change in intracellular calcium level of said cells expressing an IP-10 receptor.

17.   The method of claim 15, wherein said biological
20   response is migration of said cells expressing an IP-10 receptor.

18.   The method of claim 15, wherein said receptor is a CXCR3 receptor.
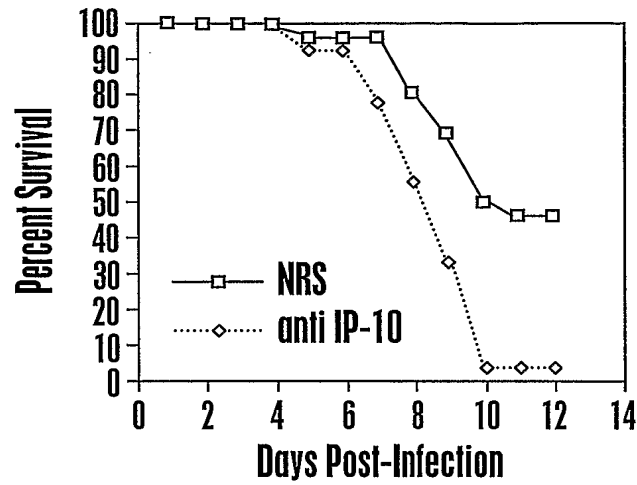
Figure 1

Treatment	Day p.i.	n[a]	Titer (pfu/g tissue, $\log_{10}$)
NRS	7	3	5.9 ± 0.1
	10	12	2.3 ± 0.4
Anti-IP-10	7	4	5.9 ± 0.1
	10	15	4.3 ± 0.8

[a] Number of mice examined. Data presented represents three independent experiments.

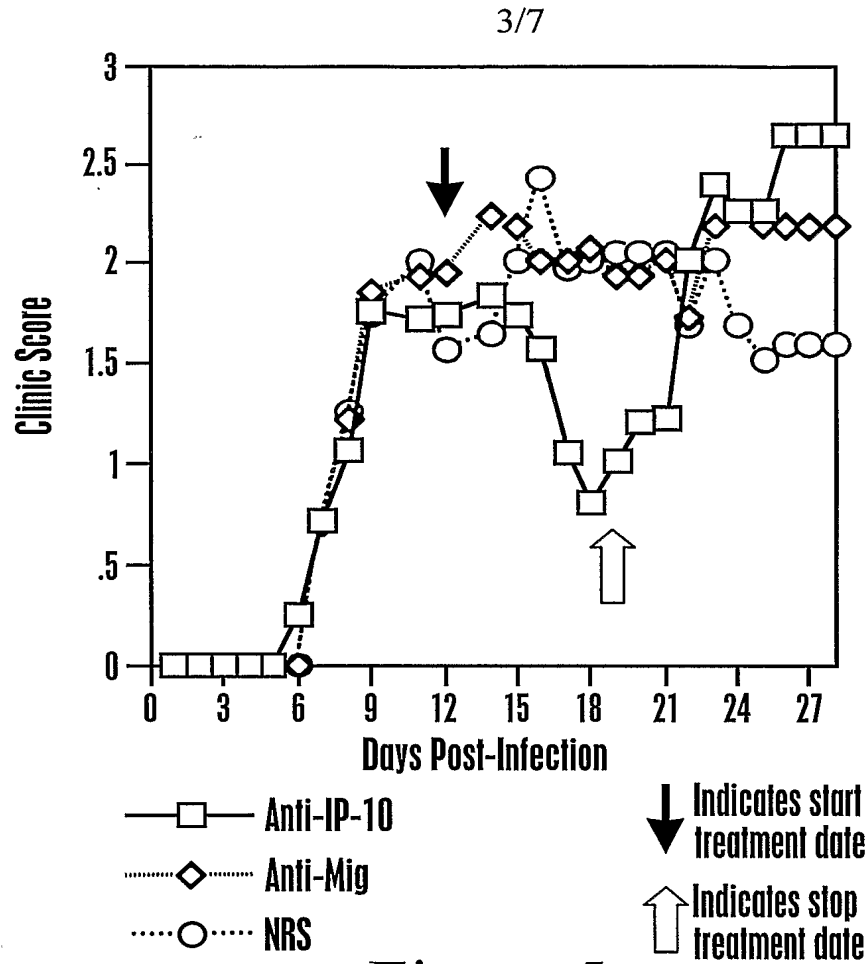**Figure 2**
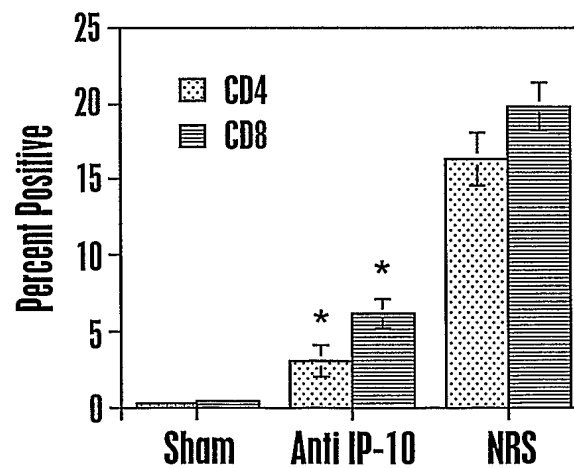
Figure 3
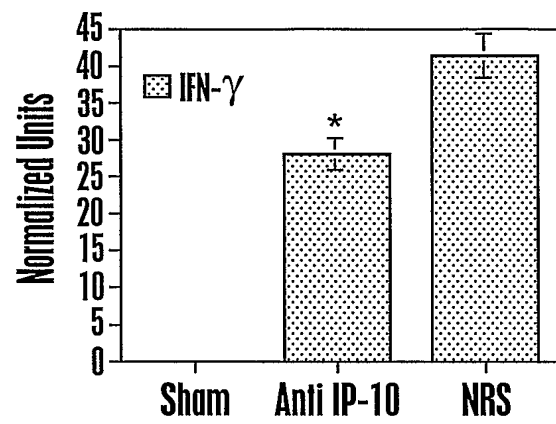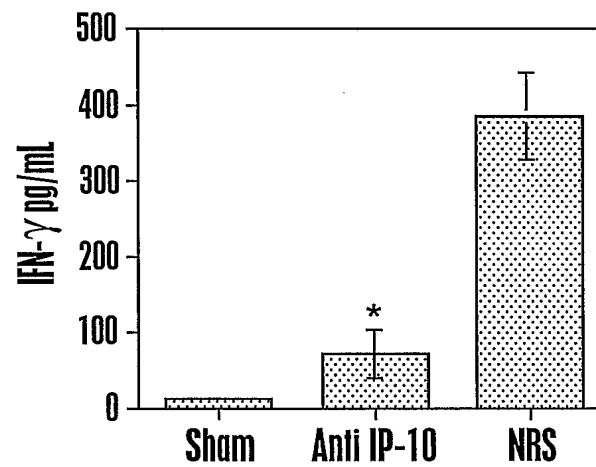
Figure 4A

Figure 4B
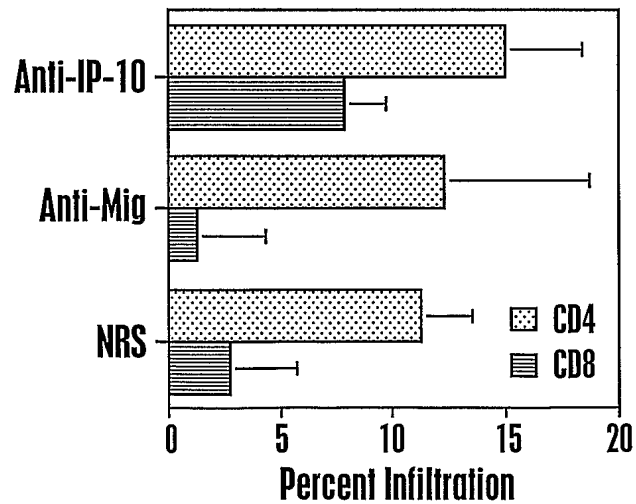
3/7
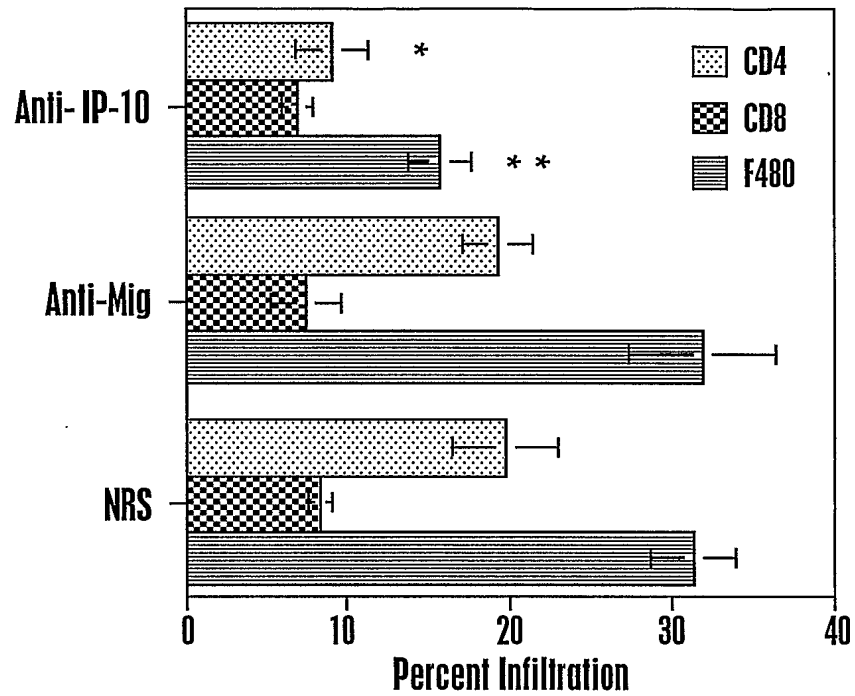
Figure 5

Figure 6

Figure 7

Treatment	PID	n	C.S. (mean + SEM)	DM (mean + SEM)
Anti-IP-10	21	3	0.50 ± 0.00	0.50 ± 0.00^a
Anti-Mig	21	4	2.5 ± 0.41	3.3 ± 0.14
NRS	21	3	2.7 ± 0.29	2.8 ± 0.17

$^a p \leq 0.001$ when compared to both anti-Mig and NRS treated mice.

Figure 8

Treatment	PID	Titer (log pfu)	n
No treatment	7	4.9 ± 0.7	8
	10	<2.0	5
NRS	7	$5.9 \pm .07$	4
	10	2.0 ± 0.2	10
Anti-Mig	7	$5.9 \pm .04$	4
	10	5.4 ± 0.2^{a}	4

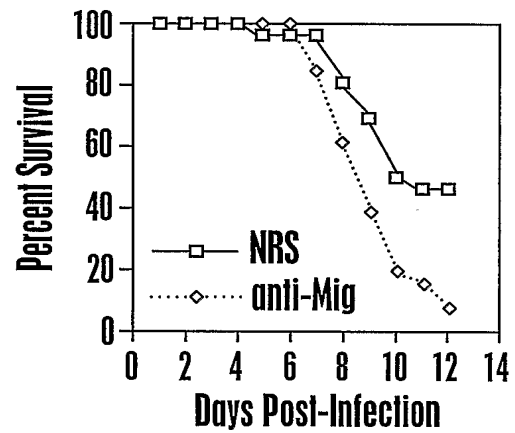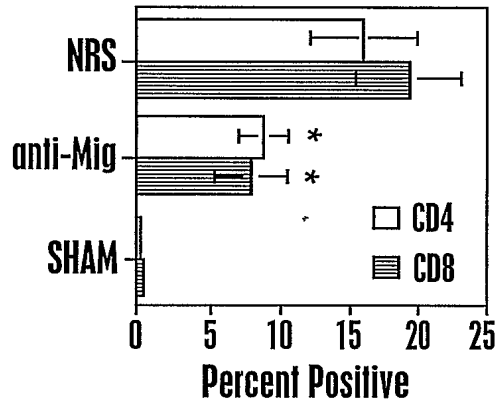$^{a}p \leq 0.001$ when compared to both anti-Mig and NRS treated mice.

Figure 9
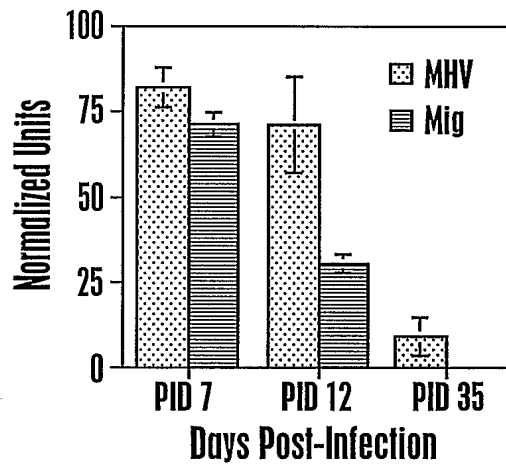
Figure 10

Figure 11

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Figure 12
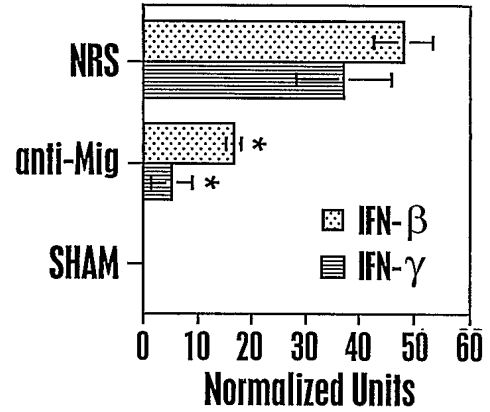

Figure 13
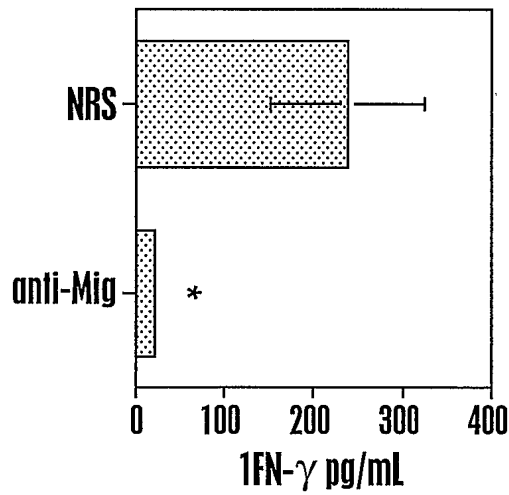
Figure 14
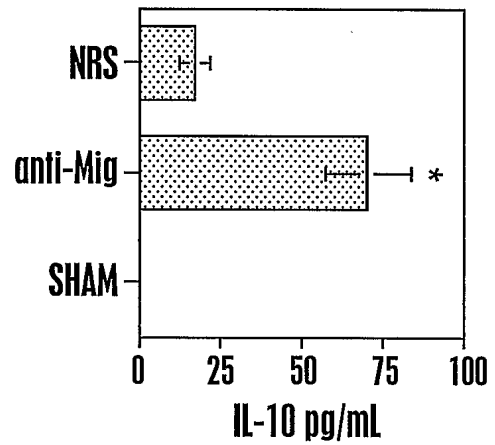
Figure 15
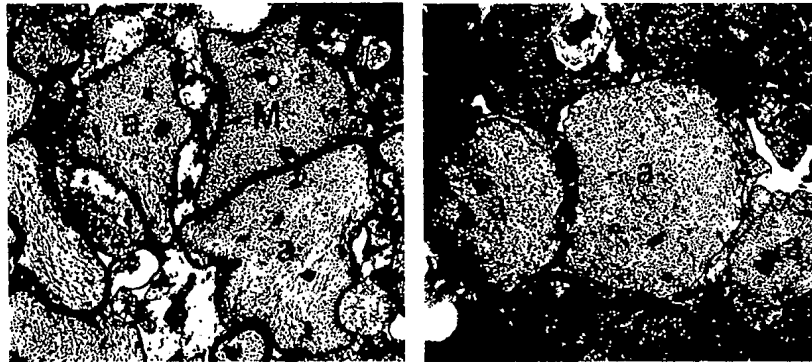
Figure 16A   Figure 16B

INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) : A61K 39/42; G01N 33/53
US CL : 424/159.1; 435/7.1
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED
Minimum documentation searched (classification system followed by classification symbols)

U.S. :   424/159.1; 435/7.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WEST, MEDLINE, CAPLUS, EMBASE, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	GANGUR, V. et al. Human IP-10 selectively promotes dominance of polyclonally activated and environmental antigen-driven IFN-gamma over IL-4 responses. FASEB J. 1998, Vol. 12. pages 705-713, see entire document.	1-18
Y	SORENSEN, T.L. et al. Expression of specific chemokines and chemokine receptors in the central nervous system of multiple sclerosis patients. J. Clin. Invest. March 1999, Vol. 103. No. 6. pages 807-815, see entire document.	1-18

| X | Further documents are listed in the continuation of Box C. | ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 JANUARY 2002	04 FEB 2002
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Authorized officer GERALD R. EWOLDT
Facsimile No. (703) 305-3230	Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet) (July 1998)*

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	BALASHOV, K.E. et al. CCR5+ and CXCR3+ T cells are increased in multiple sclerosis and their ligands MIP-1alpha and IP-10 are expressed in demyelinating brain lesions. Proc. Natl. Acad. Sci. USA. June 1999, Vol. 96. pages 6873-6878, see entire document.	1-18
Y/P	SIMPSON, J.E. et al. Expression of the interferon-gamma-inducible chemokines IP-10 and Mig and their receptor, CXCR3, in multiple sclerosis lesions. Neuropath. Appl. Neurobio. 2000, Vol. 26. pages 133-142, see entire document.	1-18
Y	TAMARU, M et al. LIVER-INFILTRATING T LYMPHOCYTES ARE ATTRACTED SELECTIVELY BY IFN-INDUCIBLE PROTEIN-10. April 2000, Vol. 12. No. 4. pages 299-308, see entire document.	1-18

DERWENT-ACC-NO: 2002-269316

DERWENT-WEEK: 200976

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TITLE: Treating demyelinating diseases and neuroinflammation comprises administering a neutralizing agent specific for interferon inducible protein 10kDa (IP-10)

INVENTOR: LANE T; LANE T E

PATENT-ASSIGNEE: UNIV CALIFORNIA[REGC] , LANE T E [LANEI]

PRIORITY-DATA: 2000US-226399P (August 18, 2000) , 2000US-226399P (August 18, 2000) , 2003US-344833 (November 12, 2003) , 2007AU-201935 (April 30, 2007)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE
WO 0215932 A1	February 28, 2002	EN
AU 200188286 A	March 4, 2002	EN
EP 1317285 A1	June 11, 2003	EN
JP 2004506696 W	March 4, 2004	JA
US 20040096446 A1	May 20, 2004	EN
AU 2001288286 B2	May 10, 2007	EN
AU 2007201935 A1	May 31, 2007	EN
EP 1317285 B1	October 7, 2009	EN
DE 60140136 E	November 19, 2009	DE

DESIGNATED-STATES: AE AG AL AM AT AU AZ BA BB BG BR BY
BZ CA CH CN CO CR CU CZ DE DK DM DZ
EC EE ES FI GB GD GE GH GM HR HU ID
IL IN IS JP KE KG KP KR KZ LC LK LR
LS LT LU LV MA MD MG MK MN MW MX MZ
NO NZ PL PT RO RU SD SE SG SI S K SL
TJ TM TR TT TZ UA UG US UZ VN YU ZA
ZW AT BE CH CY DE DK EA ES FI FR GB
GH GM GR IE IT KE LS LU MC MW MZ NL
OA PT SD SE SL SZ TR TZ UG ZW AL AT
BE CH CY DE DK ES FI FR GB GR IE IT
LI LT LU LV MC MK NL PT RO SE SI TR
AT BE CH CY DE DK ES FI FR GB GR I E
IT LI LU MC NL PT SE TR

APPLICATION-DATA:

PUB-NO	APPL-DESCRIPTOR	APPL-NO	APPL-DATE
WO2002015932A1	N/A	2001WO-US25767	August 17, 2001
AU 200188286A	N/A	2001AU-088286	August 17, 2001
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EP 1317285B1	N/A	2001EP-968007	August 17, 2001
EP 1317285A1	N/A	2001WO-US25767	August 17, 2001
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US20040096446A1	N/A	2001WO-US25767	August 17, 2001
EP 1317285B1	PCT Application	2001WO-US25767	August 17, 2001

DE 60140136E	PCT Application	2001WO-US25767	August 17, 2001
JP2004506696W	N/A	2002JP-520853	August 17, 2001
US20040096446A1	N/A	2003US-344833	November 12, 2003
AU2007201935A1	Based on	2007AU-201935	April 30, 2007

INT-CL-CURRENT:

TYPE	IPC DATE
CIPP	A61K39/42 20060101
CIPP	A61K39/42 20060101
CIPP	A61K45/00 20060101
CIPP	A61K45/00 20060101
CIPS	A61K39/395 20060101
CIPS	A61K39/395 20060101
CIPS	A61P25/00 20060101
CIPS	A61P25/00 20060101
CIPS	C07K16/24 20060101
CIPS	C07K16/24 20060101
CIPS	G01N33/53 20060101
CIPS	G01N33/53 20060101

ABSTRACTED-PUB-NO: WO 0215932 A1

BASIC-ABSTRACT:

NOVELTY - Promoting remyelination in a subject affected with a demyelinating disease and reducing the severity and reversing the neurological effects of the demyelinating disease comprises administering a neutralizing agent specific for an interferon inducible

protein of 10 kDa (IP-10).

DESCRIPTION - An INDEPENDENT CLAIM is also included for identifying a neutralizing agent specific for IP-10 comprising contacting cells expressing an IP-10 receptor with IP-10 in the presence of a candidate neutralizing agent, and determining the occurrence of a biological response associated with IP-10 activity, where non-occurrence of the biological response indicates that the candidate agent is an IP-10 neutralizing agent.

Remyelination promoter ; T-cell migration inhibitor.

USE - The method is used to promote remyelination in a subject affected with a demyelinating disease, to reduce the severity of a neuroinflammatory disease, to inhibit T-cell migration into the central nervous system (CNS) associated with a neuroinflammatory disease and to reduce the severity of demyelinating disease and impede demyelination of nerve fibres. Demyelinating diseases include multiple sclerosis, acute disseminated encephalomyelitis, acute necrotizing hemorrhagic encephalomyelitis, progressive myelopathy, Guillain-Barre syndrome, chronic inflammatory demyelinating polyradiculoneuropathy, demyelinating neuropathy associated with immunoglobulin (Ig)M monoclonal gammopathy and neuropathy associated with sclerosing myeloma. The neuroinflammatory disease includes demyelinating and non-demyelinating inflammatory diseases e.g. viral encephalitis, bacterial encephalitis and bacterial meningitis.

EQUIVALENT-ABSTRACTS:

PHARMACEUTICALS

Preferred Agent: The neutralizing agent is preferably anti-IP-10 antibody.

Preferred Method: The subject is a mammal, preferably a human. In the identifying method, the biological

response associated with IP-10 activity is a change in intracellular calcium levels or a migration of the cells. The receptor is a CXCR3 receptor.

Administration is preferably intravenous but may be topical, transdermal, intraperitoneal, intracranial, intracerebroventricular, intracerebral, intravaginal, intrauterine, oral, rectal or parenteral.

TITLE-TERMS: TREAT DISEASE COMPRISE ADMINISTER NEUTRALISE AGENT SPECIFIC INTERFERON INDUCE PROTEIN IP

DERWENT-CLASS: B04 D16 S03

CPI-CODES: B04-F01; B04-G01; B11-C07A; B11-C08E; B12-K04E; B14-A01; B14-A02; B14-C03; B14-J01; B14-J02; B14-S01; D05-H09; D05-H11;

EPI-CODES: S03-E14H4;

CHEMICAL-CODES: Chemical Indexing M1 *01* Fragmentation Code M423 M750 M781 N102 P210 P220 P420 P440 P446 P450 P510 P517 Q233 Specific Compounds RA00C8 Registry Numbers 184587

Chemical Indexing M1 *02* Fragmentation Code M423 M750 M781 N102 N136 P831 Q233 Q505 Specific Compounds RA00GT Registry Numbers 200757 200799

Chemical Indexing M2 *03* Fragmentation Code A220 C810 M411 M750 M781 N102 Q233 Q505 Specific Compounds R03033 R06646 Registry Numbers 269

Chemical Indexing M6 *04* Fragmentation Code P210 P220 P420 P440 P446 P450 P510 P517 P831 Q233 Q505 R515 R521 R614 R627 R630 R633 R639

SECONDARY-ACC-NO:

CPI Secondary Accession Numbers: 2002-079957

Non-CPI Secondary Accession Numbers: 2002-209569